CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor
EUNICE R. BROWN, Editorial Asst.
} University Farm, St. Paul 1, Minn.

CONTENTS

Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1924. Published at 500 So. 5th St., Minneapolis, Minn.

Vol. 31 **May, 1954** **No. 3**

The BAILEY #505-SS FERMENTATION CABINET

SHOWN AT THE DENVER CONVENTION

Model #505-SS

Walton Humidifier with constant level control

Stainless Exterior

Stainless Interior

Stainless or brass hardware

Built-in Sliding Shelf

110-120V 60C A.C. Current. Maximum load 1600 watts

¼ ton Refrigeration Unit
Extra – $280.00

Outside Cabinet Dimensions
60" x 60" x 24" deep

Welded steel stand is 22" high or will build to suit. Grey shrivel finish – stainless trim
Extra – $108.00

Model #505-SS Bailey Fermentation Cabinet – $1658.00
Est. Shipping Weight 1000 lbs.
Prices F.O.B. Lincoln, Nebr.

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY
LINCOLN, NEBRASKA

The Vital Story of MACARONI* ENRICHMENT

by Science Writer

This is the fourth article in a series devoted to the story of cereal enrichment

Dramatic results have been recorded about the value of enrichment in improving health. From the United States, the Bataan peninsula in the Philippines, Newfoundland and many other parts of the world comes word of the great benefits which result from enrichment.

For years, some forward-looking manufacturers of macaroni and noodle products have used enrichment to make their good foods better. They know that enrichment restores important vitamin and mineral values which are unavoidably lost in milling, and they recognize their responsibility to provide the greatest health-building benefits for the public.

Enrichment is really a simple process. It adds the following essential elements to the food during manufacture.

Thiamine—also called vitamin B_1. This vitamin helps to build physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

Riboflavin—also called vitamin B_2. This vitamin helps to keep body tissues healthy and to maintain proper function of the eyes. It is essential for growth.

Niacin—another "B" vitamin, is needed for healthy body tissues. Its use in the American diet has done much to make a serious disease called pellagra disappear.

Iron—is a mineral used in all enrichment. It is essential for making good, red blood and preventing nutritional anemia.

Products made from semolina may be enriched by two methods. One uses small square wafers which contain all the vitamins and iron necessary to enrich 100 lbs. The wafers break up in a small amount of water which is then added to the paste. For manufacturers who use the continuous press method, a powdered concentrate of the vitamins and iron, called a premix, is available. This is added by a mechanical feeding device.

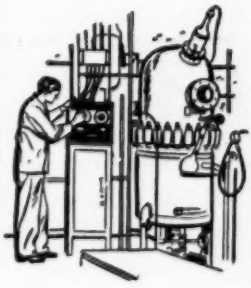
**Macaroni is used here in the generic sense. It includes all alimentary pastes: macaroni, spaghetti, pastina, noodles.*

These are the minimum and maximum levels, in milligrams per pound, required by the Federal Definitions and Standards of Identity for enriched alimentary pastes.

	Min.	*Max.*
Thiamine (vitamin B_1)	4.0	5.0
Riboflavin (vitamin B_2)	1.7	2.2
Niacin	27.0	34.0
Iron	13.0	16.5

NOTE: These levels allow for 30% to 50% losses in kitchen procedures.

Nowadays scientists are able to "build" duplicates of many of Nature's essential complexes in the laboratory. This has happened with many vitamins. First the chemical composition is learned and the pure substance is isolated. Then a "duplicate" is made which is identical chemically and biologically with Nature's product. A vitamin is a vitamin regardless of its source just as salt is salt whether it comes from a mine or is evaporated from the sea. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.



The Hoffmann-La Roche people, who produce a good percent of the vitamins used in enrichment, use amazingly complex processes with scientific production controls. This requires modern, special equipment filling whole buildings, each one a city block square and many stories high.

The combination of scientific research, thorough know-how and mammoth manufacturing processes—plus the far-sightedness of leaders in the macaroni industry—is helping vitally to make good macaroni products better.

This article, reprints of which are available without charge, is published as a service to the macaroni industry by the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West, Montreal, Quebec.

Rapid... Accurate... Reproducible...

MOISTURE DETERMINATIONS

The NEW cenco *Infrared* MOISTURE BALANCE

for
use with
such
materials as—
FLOUR
POPCORN
SORGHUM MILO
SOIL
HAY
RICE
FOUNDRY SANDS
DRY MILK PRODUCTS
OATS
PAPER
CLAY
SUGAR BEET PULP
SOYBEAN MEAL
CERAMICS
FOOD PRODUCTS

—•—

Measures either solid or liquid samples.

Here is an entirely new CENCO development which measures moisture content by weighing and drying *simultaneously*. It utilizes an improved principle of operation in drying the sample by infrared radiation and weighing by a sensitive null-point torsion balance. As a result, extremely rapid, accurate and reproducible moisture determinations are easily made.

The CENCO Moisture Balance has been used successfully for determining moisture content in a wide variety of materials. New developments now make it possible to measure liquids as well as solid samples. Disposable pans are also available at low cost for use with greasy, oily, tacky, and adhesive materials eliminating the need for cleaning.

Operation is quite simple. The scale is calibrated directly in per cent moisture from 0 to 100% in .2% divisions. Final reading indicates the percentage of moisture content.

Write for Bulletin No. 1210B.

No. 26675 Cenco Moisture Balance, $226.50 No. 26678 disposable pans for solids or liquids, Pkg. of 25, $2.20. 4 pkgs. of 25, $8.00

cenco

CENTRAL SCIENTIFIC COMPANY

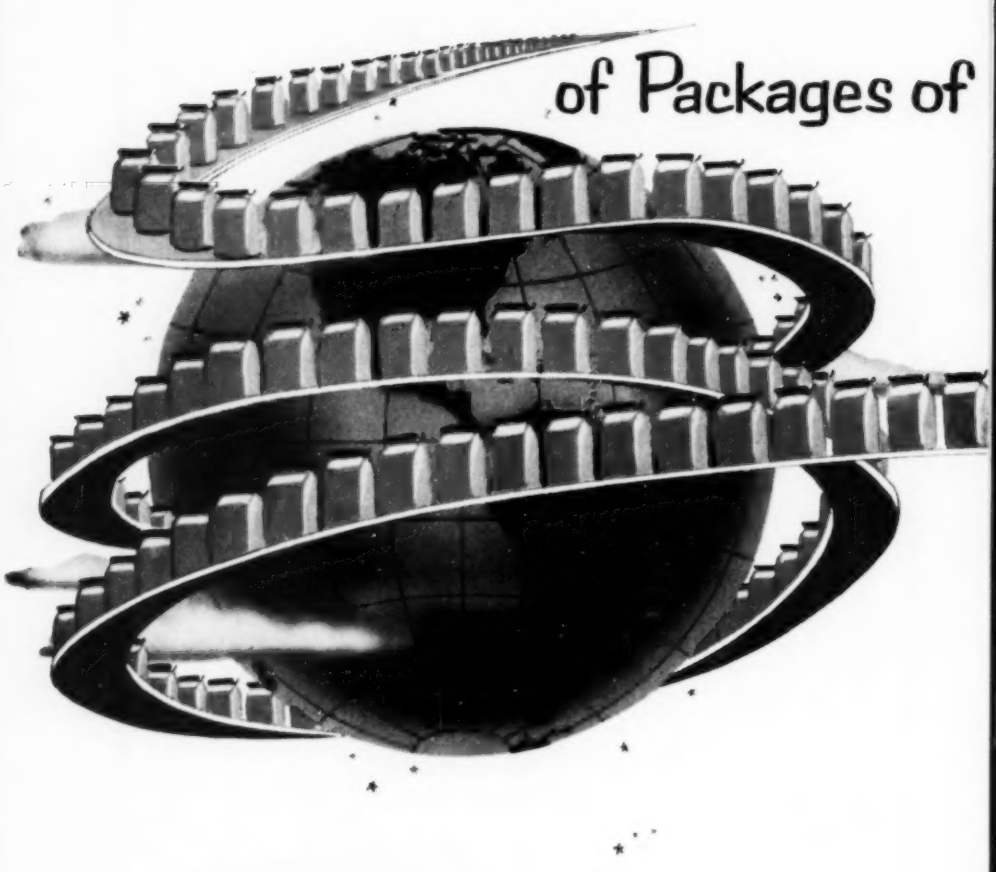
1700 IRVING PARK ROAD • CHICAGO 13, ILLINOIS

CHICAGO NEWARK BOSTON WASHINGTON DETROIT SAN FRANCISCO
SANTA CLARA LOS ANGELES TORONTO MONTREAL VANCOUVER OTTAWA

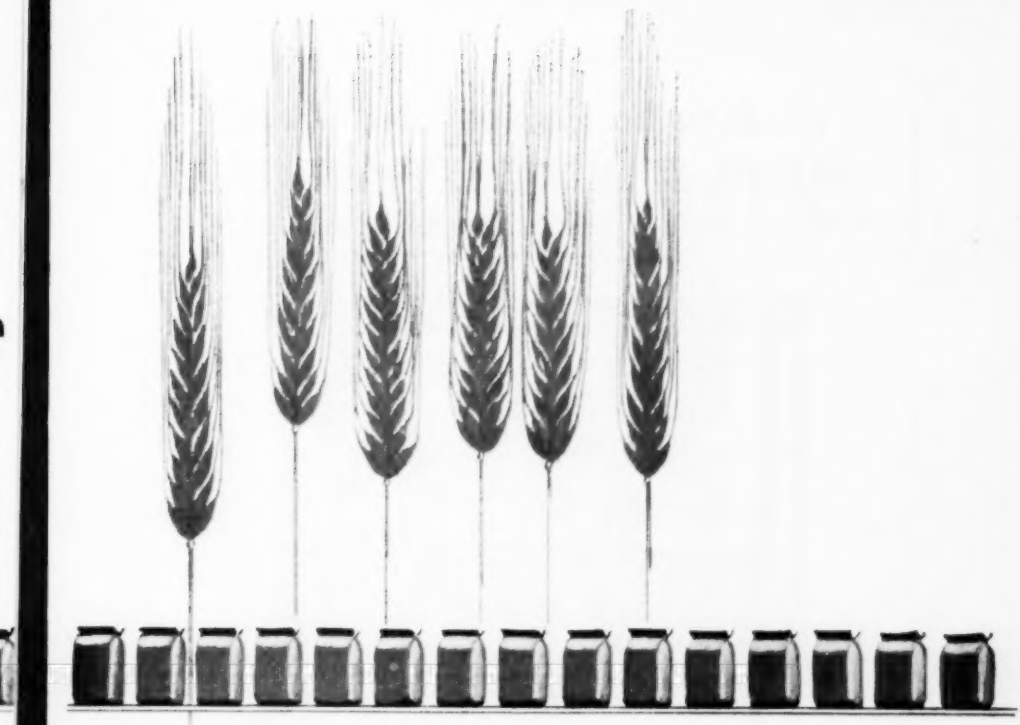
Refinery Supply Company Tube • Houston

MILLIONS

of Packages of



Self-Rising Flour-All made with V-90



what V-90's 15 years OF SHELF-LIFE EXPERIENCE means to millers . . .

There's only one way in the world to be *sure* of the stability or shelf-life of your self-rising flour. That's by *your own* actual experience in the field.

V-90's 15 years of shelf-life experience is extremely important to millers because quick tests are dependable only when based on and confirmed by actual field performance. The only reliable test is years of experience in the field under all conditions of heat and humidity. When your self-rising flour has passed this test, *you're sure!* The element of risk is eliminated.

For over 15 years, V-90 has been producing outstanding results in all kinds of pre-leavened products. To quote a major user . . . "The introduction of V-90 was the beginning of the end of the miller's troubles with self-rising flour." This is because V-90 excels not only in shelf-life but *also* in the *slower* release of leavening gas during the critical two-minute mixing period.

V-90 remains the *only* anhydrous monocalcium phosphate that the milling industry can use and *know* that baking results will always be best.

V-90®

THE ONLY PHOSPHATE CRYSTAL WITH A "RAIN COAT"®

*Coated Anhydrous Monocalcium Phosphate

VICTOR CHEMICAL WORKS

141 WEST JACKSON BLVD. • CHICAGO 4, ILL.

CEREAL CHEMISTRY

VOL. XXXI MAY, 1954 NO. 3

STALING STUDIES OF BREAD MADE WITH FLOUR FRACTIONS.

I. FRACTIONATION OF FLOUR AND PREPARATION OF BREAD[1]

W. G. BECHTEL AND D. F. MEISNER[2]

ABSTRACT

Laboratory procedures were developed for fractionating flour into gluten, starch, tailings, and solubles. Breads were baked using gluten and starch, with and without the other fractions. The 1-lb. loaves which were made were indistinguishable from bread made of the original flour.

From the early studies of the mid-nineteenth century to the present the purpose of research into the causes of the staling of bread has been to increase the time during which bread is regarded by the consumer to be fresh and palatable. The extensive literature in this field has been reviewed by Alsberg (1) and more recently by Geddes and Bice (3).

Although a number of theories have been proposed by different investigators to explain the bread-staling process, the most widely accepted have attributed staling to changes which occur in the gelatinized starch of bread (9). The evidence which has been presented for these theories has been indirect: when bread ages, it stales; when gelatinized starch ages, it retrogrades. Since no such pronounced changes have been shown to occur in other bread ingredients on aging, bread staling has been attributed to the retrogradation of starch or to one of its components, the straight or the branched-chain molecules.

The present study was undertaken to obtain direct evidence regarding the role of flour components in the staling process. It appeared probable that if breads were baked having different proportions of certain flour fractions, the properties of the resulting loaves

[1] Manuscript received November 4, 1953. The study on which this article is based was made by the American Institute of Baking under contract with the U. S. Department of Agriculture and under the authority of the Research and Marketing Act.

[2] Present address: Omar Incorporated, Omaha, Nebraska.

would show which, if any, of the fractions were responsible for staling. Such evidence could not be obtained by use of a number of flours of different gluten and starch proportions. If different flours were used, not only would the proportion of starch and gluten vary but their biological nature would differ also, since they would be produced from different wheats or by different combinations at the mill. That the baking properties of gluten and starch from different flours do vary in a pronounced manner has been shown by Sandstedt, Jolitz, and Blish (8), Harris and Sibbitt (5), and others. The method employed in these studies was to fractionate a single flour, bake breads using different proportions of the fractions, and study the staling characteristics of the resulting products by means of sensory tests.

Materials and Methods

The flour was a commercial baker's patent spring wheat flour which was bleached and bromated, but not enriched. Its protein content was 12.6% and ash was 0.47% at 14% moisture.

Flour Fractionation. Flour was separated into four fractions: gluten, "prime-quality" starch (hereafter referred to simply as starch), tailings starch (referred to as tailings), and solubles. The method of separation employed was not new in principle, although well-known methods were modified to meet the conditions imposed by available equipment.

Flour, 12 lb., and water, 9 lb., at 45°–50°F. were mixed to a dough in a Hobart model M800 mixer using a 20-gal. bowl and dough hook. Mixing was 2 minutes at speed 1 and 8 minutes at speed 2. Immediately after the dough was mixed 11 lb. of water were added and mixed for 5 minutes at speed 1. The major part of the dough was separated from the aqueous starch and tailings suspension. The latter was then filtered through a fine-mesh cloth to remove the remaining gluten particles. The dough was replaced in the mixer and washed with 22 lb. of water for 10 minutes at speed 1. Starch and tailings were separated from the dough as before. This process was then repeated twice.

The gluten was immediately cut into pieces of about 170 g., wrapped in waxed paper, placed in press-top cans and frozen and stored at −28°C. (−18°F.) until needed. The avgrage analysis was 65.0% water and 70.1% protein, dry basis. Variation of individual batches was ±1.5% for water and ±2% for protein. The aqueous solution containing suspended starch and tailings was refrigerated overnight at 38°–40°F. to permit the suspended solids to settle. As much as possible of the liquid was removed by decantation. The starch

and tailings were resuspended and centrifuged. Attempts to separate the tailings from starch in a basket centrifuge failed, as the fractions were more or less mixed. This agreed with the observation of Clendenning and Wright (4). In the absence of a continuous centrifuge of large capacity, the suspension was centrifuged in jars of extra wide mouth to permit easy removal of contents. By this process a compact layer of starch was formed, above which was a well-defined layer of tailings.

The starch was separated from the tailings and dried in air to about 10% moisture. It consisted almost entirely of large, undamaged starch granules. Its protein content was 0.1% and the pentosan content was 1.8% dry basis. It was passed through a 20-mesh sieve while still slightly damp. The resulting particles were sufficiently fine to disintegrate readily in water.

The tailings fraction, which contained about 80% moisture, dried in air very slowly to form a hard, horny mass. It was possible to dry it much more rapidly by mixing it with three volumes of acetone or 95% alcohol. This caused a separation of solids which could then be filtered by suction. The product treated in this manner was then dried in air. It was necessary to grind it to pass a 50-mesh sieve to insure its dispersion in dough. No difference was found in the swelling power of the air-dried and the acetone-treated products. Both were suspended in water at 10% concentration with continuous stirring for 4 hours, after which they were centrifuged and the residue was weighed. The water absorbed in each case was 2.3 times the weight of the dry tailings. Since no difference could be detected between bread made with the two products, and partial dehydration with acetone increased the speed of drying, most of the product was prepared in this manner. Various names have been proposed for the tailings fraction. Sandstedt, Jolitz, and Blish (8) called it "amylodextrin" fraction. MacMasters and Hilbert (6) suggested the term "tailings" fraction, and Clendenning and Wright (4) referred it to as "squeegee" starch. While its composition when dry is about 90% starch, consisting largely of the small and damaged granules, it contains a relatively large amount of pentosans. Complete analysis of this fraction by MacMasters and Hilbert (6) showed that it consisted of starch, pentosans, fat in about the same proportion as in wheat starch, cellulose, and ash. Analysis of the tailings fraction used in these experiments gave the following results: protein 1.6%, pentosan 7.4%, dry basis.

In the fractionation of each 12 lb. of flour a total of 86 lb., or about 10 gal., of water was used. This dilute solution of solubles was concentrated to about 15.2% solids, using a circulating evaporator built ac-

cording to the general description of Mitchell, Schildneck, and Dustin (7). A tinned copper heating tube and a copper condenser were used, and the receiving flask was surrounded by ice. Steam was used for heat. About 0.5 gal. of water was evaporated per hour at a liquid temperature of 30°C. (86°F.) and a vacuum of 28.5 inches of mercury, obtained by use of a water aspirator. Each charge of liquid in the evaporator was removed within 3 to 4 hours. It was then frozen and stored at −28°C. When a sufficient quantity of concentrate was obtained, it was melted, thoroughly mixed to insure uniformity, and stored at −28°C. until required. Analysis of the dry solids gave protein 14.6%, and pentosans 11.7%. Complete analyses of the fractions were not made, since there is no reason to believe that they would differ materially from the published analyses of other investigators who used similar separation procedures.

Analytical Methods. Protein was analyzed by the Kjeldahl method (N × 5.7). For pentosans the A. O. A. C. phloroglucinol method was used (2).

Baking of Bread. A considerable amount of experimentation was required before 1-lb. loaves of a quality similar to that of commercial baker's bread could be produced. As the result of systematic studies of mixing time, fermentation time, and kinds and proportions of ingredients, the following baking procedure was developed, using a sponge-dough process with a 50% sponge.

	Sponge	*Dough*
Gluten, frozen	(a)	(a)
Wheat starch and tailings	(a)	(a)
Water	(b)	(b)
Yeast (c)	2%	...
Yeast food (d)	1%	...
Rhozyme 33 (e)	0.04%	0.04%
Salt (f)	...	2%
Sugar, sucrose	...	6%
Non-fat dry milk solids	...	4%
Lard	...	2%

A Hobart Model A-120 mixer with McDuffee bowl and fork was used. Mixing time for both sponge and dough was 3 minutes at speed 1 followed by 3 minutes at speed 2. Sponge fermentation time was 4 hours. Doughs were given no floor time, an intermediate proof of 12 minutes, and were proofed to height in the pan. Loaves were scaled at 18½ oz. and were baked 23 minutes at 450°F.

(a) Gluten and starch were used in the desired proportion to produce 650 g. "flour" of 14% moisture. Bread has been prepared using "flours" containing from 8.5% to 26% protein.

(b) The amount of water to be added as such to sponge and dough depends on the proportion of gluten and the nature of the starch component. Since the gluten is about two-thirds water, a considerable part of the requisite water is added with it. Starch has a lower absorption than tailings. The absorption was adjusted on the basis of experimental baking tests to give bread of the desired quality.

(c) Percentages of ingredients are based on the total weight of starch, tailings, and gluten at 14% moisture.
(d) Yeast foods containing buffering salts offered no advantage.
(e) Manufactured by Rohm and Haas Co., Philadelphia.
(f) In bread of 17.2% flour protein the salt was increased to 2½% to improve flavor.

Water, gluten, and the dry ingredients were added to the bowl and after 3 minutes of mixing the starch which had failed to become incorporated in the dough was loosened from the bottom of the bowl. During the 3 minutes of rapid mixing a smooth dough formed.

Results

The fractions obtained from flour and the proportion of each were as follows, calculated on the basis of dry flour:

	%
Gluten	13.4
Starch	59.1
Tailings	13.9
Solubles	7.6
Loss	6.0

The losses were mechanical. Some gluten particles adhered to the filter used for starch separation, while a small amount of starch was unavoidably lost in each operation until it was dried.

Breads produced by recombining one or more of the other fractions of flour with gluten were similar to commercial baker's bread in external and internal characteristics when the "flour" contained between 10.5% and 17% protein. The breads shown in Fig. 1 are typical of those made of the flour fractions gluten and starch only. Other breads, made by use of gluten and mixtures of (a) 10% tailings and 90% starch, and (b) 20% tailings and 80% starch, were similar in characteristics. Bread made by use of solubles in addition to starch and gluten was somewhat more dense, but still compared favorably with commercial loaves of low specific volume.

The crumb of the bread made of "flour" containing 8.6% protein was coarse and open, and the loaf volume was lower than those of higher gluten content. "Flour" containing 10.6% protein gave bread, not illustrated, very similar to that of "flour" containing 12.7% protein. When the flour protein content was raised much above 17.2%, as in breads 4 and 5, loaf characteristics remained good but the flavor became inferior and was described as glutenlike, while the crumb became very gummy, a property retained to a large degree for the 5 days during which the bread was observed.

Discussion

Several investigators have prepared bread from gluten and one or more of the other fractions of flour. Sandstedt, Jolitz, and Blish (8)

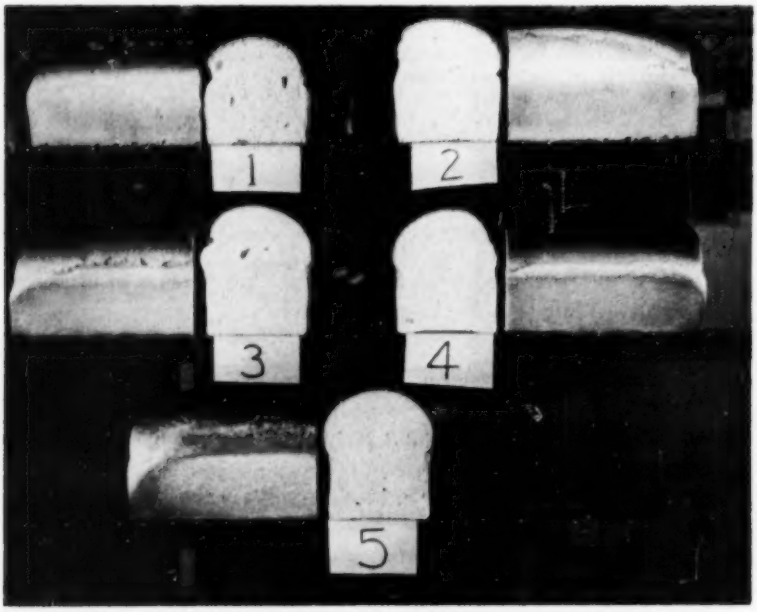


Fig. 1. Typical breads made with gluten and starch as the only flour components. Percentage of flour protein, on basis of total gluten and starch at 14% moisture: No. 1, 8.6%; No. 2, 12.7%; No. 3, 17%; No. 4, 21.5%; No. 5, 25.8%.

used such bread to investigate the baking properties of starches, while Harris and Sibbitt (5) studied starches and glutens. In such studies small-size loaves were prepared, and interest centered on loaf volume and, in some cases, dough and crumb characteristics. The problem in the present study was very different for, to use the bread for sensory test of staling, it was necessary to prepare full-size loaves. Not only was it necessary for loaf and crumb characteristics to be satisfactory, but the flavor had to match that of baker's bread closely enough so that panel members were unaware that the bread samples were in any way peculiar.

In the developmental work and the staling tests which followed, more than 500 1-lb. loaves of bread were prepared and used. It was because of this large-scale requirement for flour components that the fractions described above were used, rather than more completely separated chemical entities.

During preliminary developmental experiments, small-size loaves were baked to save materials. A procedure was perfected which yielded loaves of good volume and crumb characteristics. However, when the attempt was made to use this procedure to prepare 1-lb. loaves, the

internal characteristics were poor and the volume low, necessitating the development of an entirely new baking procedure.

Straight-dough procedures which were tried failed to yield bread of good quality. In experiments with the sponge-dough method, sponges with different proportions of gluten, from 100% to 50% of the total, were used. The best bread was obtained with a 50% sponge. Preliminary work proved that yeast food was required and 1%, based on the "flour," was found to be the optimum amount.

It seems probable that in the process of fractionating flour part of the enzymes would be found in the solubles fraction. When bread was baked without using the solubles and without addition of enzymes the loaves were flat and of low volume, and the crumb was dark and coarse, with thick cell walls. Addition of barley malt flour improved the quality of the bread as the quantity was increased up to 1% of the "flour." Malt flour at 2% gave a loaf of inferior volume and coarse, soggy crumb. No level of malt was found which gave bread of superior quality. Rhozyme 33 is a fungal enzyme preparation of high alpha-amylase activity. As the result of baking tests using Rhozyme 33 at various levels in the sponge, it was found that 0.04% based on the "flour" gave bread of greater volume and much better crumb characteristics than were obtained with any level of malt. When an equal amount of Rhozyme 33 was added to the dough also, there was a further improvement in volume, and the crumb characteristics were excellent. The alpha-amylase added in the fungal enzyme was approximately 8 times that which produced the best loaf with malt. Rhozyme 33 is relatively low in proteolytic activity. It appears likely that the great improvement in the bread, brought about by the use of the large excess of this fungal amylase, was due to the proteolytic enzyme it supplied in sufficient amount to soften the gluten properly. Because of the low thermal stability of fungal amylase this could be accomplished without overdiastating the dough, whereas, when malt was employed, the dough was overdiastated before a sufficient amount of protease could be introduced.

Sugar at 4% of the "flour" was ample for good fermentation. A great improvement in flavor resulted when the proportion of sugar was increased to 6%. In bread of "flour" containing 17.2% protein, the proportion of salt was raised to 2½% to improve flavor. This minor change in formula did not noticeably affect any other property.

Loaves of bread made with gluten and starch as the only "flour" components, those made with gluten, starch and tailings fractions (with tailings at 10% and 20% of the total starch-tailings mixture), and those with all of the fractions of flour were distributed for ap-

praisal and were accepted as ordinary baker's bread. When used for sensory tests of staling, panel members also accepted the samples as being ordinary bread.

Acknowledgment

The authors wish to express their appreciation to Mr. George Hammer of the experimental bakery of the American Institute of Baking, for baking and scoring the breads, and for his helpful suggestions during the development of baking procedures.

Literature Cited

1. Alsberg, C. L. The stale-bread problem. Wheat Studies of the Food Research Institute **12:** 221–247 (1936).
2. Association of Official Agricultural Chemists. Official and tentative methods of analysis (6th ed.), p. 412. The Association: Washington, D.C. (1945).
3. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives (3rd ed.), by J. A. Radley; Vol. II, Chap. 10. Chapman and Hall: London (1953).
4. Clendenning, K. A., and Wright, D. E. Separation of starch and gluten. V. Problems in wheat starch manufacture arising from flour pentosans. Can. J. Research F **28:** 390–400 (1950).
5. Harris, R. H., and Sibbitt, L. D. The comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Cereal Chem. **19:** 763–772 (1942).
6. MacMasters, M. M., and Hilbert, G. E. The composition of the "amylodextrin" fraction of wheat flour. Cereal Chem. **21:** 548–555 (1944).
7. Mitchell, D. T., Schildneck, P., and Dustin, J. Laboratory-size glass circulating evaporator. Ind. Eng. Chem. (Anal. Ed.) **16:** 754–755 (1944).
8. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. **16:** 780–792 (1939).
9. Schoch, T. J., and French, D. Studies on bread staling. I. The role of starch. Cereal Chem. **24:** 231–249 (1947).

STALING STUDIES OF BREAD MADE WITH FLOUR FRACTIONS.

II. SELECTION OF THE SENSORY TEST PANEL[1]

W. G. Bechtel and D. F. Meisner[2]

ABSTRACT

A group of volunteers was trained in observing the changes which occur in bread as it ages. This group was then given a series of rating scale tests in judging the freshness of bread which was from 1 to 6 days old. After several replications of the test the results of each trainee were analyzed statistically, to estimate the ability of the individuals to discriminate between samples of different age, and to judge samples of the same age consistently. For this purpose the F-value, the ratio of the variance between samples to that within samples, was used. Those with highest F-values were accepted as panel members, eliminating all with F-values below that required for the 5% level of confidence.

It has been shown by Bice and Geddes (3), Bechtel and Meisner (1), and Bechtel, Meisner, and Bradley (2) that results of the physical laboratory tests which have been used by many investigators to study the staling of bread do not always correspond with judgments of bread freshness made by human observers. This has led these investigators to the conclusion that changes in crumb compressibility or firmness, crumbliness, swelling power, and the amount of so-called soluble starch or of its fractions are not reliable measures of the staling process, and that sensory tests afford the only reliable means for studying staling changes.

In previous investigations of the staling phenomenon in this laboratory a large, randomly-selected sensory panel of 90 to 95 members was used. It was believed that with such a large number of observers reliable results could be obtained without selection of individual members on the basis of superior acuity and reliability of judgment. Use of a large panel for the present study was undesirable because of the great amount of bread which would be consumed for each test. This presented no problem so long as the bread was made of flour, but it was not practical to prepare flour fractions in sufficient quantity to bake bread for the large panel. A smaller panel appeared attractive also, because of the greater ease of administering the tests and analyzing the results. With a small panel it became necessary to train and select the members to insure that they would be capable of detecting

[1] Manuscript received November 4, 1953. The study on which this article is based was made by the American Institute of Baking under contract with the U. S. Department of Agriculture and under the authority of the Research and Marketing Act.

[2] Present address: Omar Incorporated, Omaha, Nebraska.

differences in freshness of bread, and that they would judge replicate samples to be of the same freshness.

Methods

Panel Training. A preliminary group of volunteers, considerably larger than the desired panel, was trained in judging bread freshness. Discussions were conducted during which the factors involved in the judgment of freshness (4) were illustrated and explained. The factors discussed were the changes in the following properties:

Feel of the crumb of a cut slice to the fingers. This involves the firmness of the crumb and the harshness or smoothness of the cut surface. As bread stales the crumb becomes firmer and harsher.

Odor and flavor. The odor and flavor of fresh bread disappear on staling and a less pleasant odor and flavor appear.

Feel of bread in the mouth. Fresh bread feels moist, and the crumb is cohesive. As it stales it feels dry and crumbly in the mouth.

It was made clear that preference must not enter into the judgment, and that the sole problem was to determine the degree of freshness or staleness of the samples.

Name_____ Date_____

Open one sample bag, judge the sample by feel in the fingers, odor, flavor, feel in the mouth, or in any manner that you ordinarily judge the freshness of bread. Write the sample number at the top of the first column, and place a check opposite the term which best describes the sample. Then proceed to the second sample in similar manner. Continue to judge each sample without comparison with the others.

Sample No.				
Very fresh				
Fresh				
Slightly fresh				
Slightly stale				
Stale				
Very stale				

Fig. 1. Rating scale for staling studies.

Rating Scale. In both panel-selection tests and staling studies a six-point rating scale was used, as shown in Fig. 1. The rating scale technic was adopted because it would give information about the magni-

tude of the differences between samples, whereas such methods as paired comparison supply only information as to whether a difference exists. A six-point scale was selected as being sufficient to include an adequate number of ratings to differentiate clearly between samples of different degrees of freshness without being so extensive as to complicate judgments unduly. Descriptive terms were selected so that an equal number would suggest degrees of freshness and staleness, and so that corresponding terms in the two halves of the scale would suggest the same degree of difference on each side of the center. For example, it was expected that the term *very fresh* would carry emphasis equivalent to that of *very stale*.

Testing Method. The number of samples given on any one day was usually three, although occasionally two or four were used. Samples were selected so that there was no relationship between the day of the test and the age of the samples. For example, on the second consecutive day of testing, the samples were never one day older than on the preceding day. They might be fresher, or considerably older. Likewise samples given the same day did not differ in age by a uniform amount. All tests were given under uniform conditions of lighting, temperature, and physical surroundings, and at the same time of day. Samples consisted of a half-slice of bread cut vertically, ½ in. thick. Uniformity of thickness was assured by cutting the slices in a miter box. Samples were packaged immediately after slicing, in bags of moistureproof Cellophane which were then heat-sealed. Each sample was coded with a two-digit number selected by chance, and each bag carried the sample number. Panel members were informed that the code was random and bore no key to the kind or age of bread.

Panel Selection. Members of the staling panel were selected from the trainees on the basis of superior ability to discriminate between samples of different ages, and reliability in giving replicate samples the same rating. Trainees were given a series of rating scale tests in which they judged the freshness of white bread after storage periods of from 1 to 6 days. Several replications were made at intervals up to 4 weeks.

In order to make a statistical analysis of the judgments of each individual, the ratings of Fig. 1 were given consecutive numerical values from 1 for *very stale* to 6 for *very fresh*. The analysis of variance was applied to these data as suggested by Girardot, Peryam, and Shapiro (5). To obtain the variance between samples and between replicates, the technic described by Youden (6) was used.

The F-value, the ratio of the variance between samples to that within samples, was used to estimate the ability of individuals to dis-

criminate between samples of different age, and to judge the same sample consistently. Those with highest F-values were accepted as panel members, eliminating all with F-values below that required for the 5% level of confidence.

Results

The panel for one study of bread freshness was selected from a group of 36 individuals. Of these, 12 had F-values above that required for the 1% level of confidence, an additional 13 had F-values above that required for the 5% level, and 11 were eliminated. The 20 members with highest F-values were taken as the panel, while the remaining five who qualified were taken as alternates. Judgments of the alternates were used in the absence of panel members, thus keeping the panel at 20 members for all tests.

In a second study 27 individuals were given the panel-selection test. Of these, 12 had an F-value above that required for the 1% level of confidence, and six others qualified with an F-value above that required for the 5% level. The 16 with highest F-values were selected as panel members with the remaining two as alternates.

Discussion

It is easy to recognize and rate bread which is very fresh. Other stages are much more difficult to discriminate and to place reliably on the rating scale. Most difficult are the intermediate positions *slightly fresh* and *slightly stale*. In Fig. 2 the rating scale judgments of two of the candidates are given. One, selected as a panel member, had an F-value of 12.4, well above that required for the 1% level of signif-

RATING SCALE JUDGMENTS

Scale values: 6, very fresh; 5, fresh; 4, slightly fresh; 3, slightly stale; 2, stale; 1, very stale.

Age of Bread (hours)	Panel Member F-Value 12.4					Rejected F-Value 1.05				
	Scale Values					Scale Values				
20	6	6				4	6			
44	5	5				6	5			
68	3	4	4			5	5	4		
92	4	4				3	5			
116	3	4	2	2	3	4	2	5	5	4
140	1	2	3	1		3	3	5	4	

Fig. 2. Examples of results of those selected and rejected for use as panel members.

icance. While there are some differences in judgment of bread of the same age, it may be seen by inspection that scores of replicate samples agree well and that there is a decrease in the scores as the bread aged. The other, rejected for the panel, showed great variability in judgment of replicates even on bread 20 hours old, and failed to find much change as the bread aged.

Literature Cited

1. Bechtel, W. G., and Meisner, D. F. Present status of the theory of bread staling. Food Technology **5:** 503–505 (1951).
2. Bechtel, W. G., Meisner, D. F., and Bradley, W. B. The effect of the crust on the staling of bread. Cereal Chem. **30:** 160–168 (1953).
3. Bice, C. W., and Geddes, W. F. Studies on bread staling. IV. Evaluation of methods for the measurement of changes which occur during bread staling. Cereal Chem. **26:** 440–465 (1949).
4. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives, by J. A. Radley (3rd ed.), Vol. II, Chap. 10. Chapman and Hall: London (1953).
5. Girardot, N. F., Peryam, D. R., and Shapiro, Ruth. Selection of sensory testing panels. Food Technology **6:** 140–143 (1952).
6. Youden, W. J. Statistical methods for chemists, pp. 29–32. John Wiley & Sons: New York (1951).

STALING STUDIES OF BREAD MADE WITH FLOUR FRACTIONS.

III. EFFECT OF CRUMB MOISTURE AND OF TAILINGS STARCH[1]

W. G. Bechtel and D. F. Meisner[2]

ABSTRACT

Sensory tests of staling and laboratory tests of physical properties of bread were made at intervals throughout a 6-day storage period. After equal storage periods bread of higher crumb moisture content was judged to be significantly fresher than bread of lower moisture content. These results were obtained both with bread made of flour and with that made from flour fractions.

When made with the same moisture content there was little difference in the staling rate of bread made from flour fractions as the proportion of tailings fraction of flour was increased. Tailings fraction may, however, be of importance in the staling of bread made of flour. The tailings fraction has a higher moisture absorption than wheat starch, thus its presence in flour permits the use of more water in the dough. This in turn yields bread of higher moisture content than if the tailings fraction were absent.

The effects of changes, either demonstrated or hypothetical, in gluten and starch on the staling of bread have been subjects of a great deal of research and speculation during the past century (4). Little, if any, attention has been given to the possible effects of the tailings starch fraction of flour. Similarly, since the time of Boussingault, little attention has been given to the possibility of a relationship between crumb moisture and staling. These studies were undertaken to investigate the effect of the tailings fraction of flour, and of crumb moisture on sensory tests of bread staling.

To test the effect of tailings starch, full-sized loaves of bread were baked by the methods described by Bechtel and Meisner (1), using the flour fractions gluten, starch, and tailings, in place of flour. Bread could not be baked with gluten and tailings as the only flour components. When the starch component used in the bread contained 40% tailings and 60% starch, the crumb was very wet and soggy, and the crumb structure was so weak that in some cases the sides of the loaves caved in on cooling. When the tailings fraction was reduced to 20% of the total starch the resulting bread was of good volume and had good external and internal characteristics. This was approximately the ratio of tailings to total starch obtained from flour (1).

[1] Manuscript received November 4, 1953. The study on which this article is based was made by the American Institute of Baking under contract with the U. S. Department of Agriculture and under the authority of the Research and Marketing Act.

[2] Present address: Omar Incorporated, Omaha, Nebraska.

Moisture absorption of tailings is much greater than that of the starch fraction (5). Therefore it was not possible to bake bread of the same moisture content using in one case only gluten and starch, and in the other, gluten and a mixture of 20% tailings and 80% starch. It was found that breads of the same moisture content could be made using gluten and starch only, and gluten and a mixture of 90% starch and 10% tailings. Also satisfactory breads of the same moisture content could be made using in one case gluten and a mixture of 20% tailings and 80% starch, and in the other gluten and a mixture of 10% tailings and 90% starch. The effect of crumb moisture was studied by baking three breads of the same composition except for the amount of water used to make the dough. Because of the general belief that staling is not related to crumb moisture and its changes, additional sensory staling tests were conducted on breads, of different moisture contents, made of flour.

Materials and Methods

Flour Fractions. The flour fractions used in the breads for this study were separated by the method described by Bechtel and Meisner (1).

Baking Procedure for Bread Made of Flour Fractions. The procedure has been given (1) in terms applicable to all bread prepared from flour fractions. For the study of the effects of moisture and tailings starch the quantities not specified in the general procedure were as follows:

Bread No.	*1*	*2*	*3*	*4*	*5*
	g.	*g.*	*g.*	*g.*	*g.*
Gluten (a)	342	342	342	342	342
Starch (b)	510	510	510	459	408
Tailings (b)	0	0	0	51	102
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
Water in sponge (c)	82	82	82	105	105
Water in dough (c)	15	55	90	100	100

(a) Gluten was stored frozen without being dried. Amounts given above are for gluten of 65% water and 70.1% protein, dry basis. Different batches varied a small amount in moisture and protein. The amounts of gluten, starch, and tailings specified yield 650 g. "flour" of 12.9% protein on a 14% moisture basis. This was sufficient for two 1-lb. loaves of bread. Fifty percent of the starch, tailings, and gluten was used in the sponge.

(b) Calculated to 14% moisture.

(c) A considerable proportion of the requisite water was contained in the gluten.

Baking Procedure for Bread Made of Flour. Bread was made by the sponge-dough process, using the following formula:

	Sponge %	*Dough* %
Flour	60	40
Water (a)	..	..
Yeast	2	..
Arkady	0.5	..
Malt flour (b)	..	..
Salt	..	2
Sugar (sucrose)	..	4
Nonfat dry milk solids	..	4
Lard	..	2

(a) Absorption, bread of higher moisture content, 68%; bread of lower moisture, 58%.
(b) As determined by the amylograph to give a maximum reading of 500 Brabender Amylograph units when 100 g. flour were suspended in buffered distilled water to a total of 450 ml. at pH 5.35, and heated at the rate of 1.5° C. per minute.

Bread Storage. Bread was sealed in waxed paper and stored at 75°F.

Sensory Tests. These were made by the method described by Bechtel and Meisner (2) using the trained panel of 16 members selected for superior ability to distinguish between bread of different ages, and for reliability in judging replicate samples.

Laboratory Tests. Methods for moisture and compressibility have been described (3). One loaf of each batch was tested for compressibility and moisture while the other was used for sensory tests, thus insuring that panel and laboratory tests were made on loaves of identical properties.

Results

The effect of a change in crumb moisture on the staling of bread, made with gluten and starch as the only "flour" constituents, is shown in Table I. Bread 1 was approximately 2% lower in crumb moisture than bread 2. In each sensory test during the 6-day study, bread 1 was judged less fresh than bread 2 of the same age. Bread 3 was approximately 2% higher in crumb moisture than bread 2, and on each day of the study it was judged by the panel to be fresher than bread 2 of the same age. Thus there was found to be a direct relationship between crumb moisture and judgment of freshness.

The statistical significance of the results is given in Table I. In all but two of the tests the bread of higher crumb moisture was judged significantly fresher than that of next lower moisture. The level of confidence of the differences in freshness between breads 1 and 3, representing the extremes in crumb moisture, was below 0.1% except for the test at 20 hours.

The effect of a difference in crumb moisture on the judgment of freshness of bread made with flour is shown in Table II. On each day

TABLE I

EFFECT OF DIFFERENCES IN CRUMB MOISTURE ON THE FRESHNESS OF BREAD MADE OF GLUTEN AND WHEAT STARCH

Bread No.	Bread Property	Time of Storage (Hours)				
		2	20	68	116	140
1	Crumb moisture,	37.1	...	...	...	32.4
2	percent[a]	39.0	...	...	...	33.5
3		41.0	...	...	...	36.2
1	Compressibility,	...	63	28	13	12
2	mm. × 10	...	81	37	16	25
3		...	96	42	31	25
1	Panel judgment	...	4.69	2.19	1.81	2.31
2	of freshness[b]	...	5.31	2.38	2.38	2.94
3		...	5.50	3.50	3.63	3.50
1	Variance in	...	0.71	0.53	0.78	0.46
2	panel judgment	...	0.24	0.73	0.51	0.68
3		...	0.25	0.75	0.48	0.50
1 & 2	Significance	...	*	none	*	*
2 & 3	of freshness	...	none	***	***	*
1 & 3	difference[c]	...	**	***	***	***

[a] Moisture loss from a loaf of 450 g. was 1.1±0.5 g. in 6 days.
[b] Based on the rating scale: 6, very fresh; 5, fresh; 4, slightly fresh; 3, slightly stale; 2, stale; 1, very stale.
[c] * 5% level; ** 1% level; *** 0.1% level.

TABLE II

EFFECT OF A DIFFERENCE IN CRUMB MOISTURE ON THE FRESHNESS OF BREAD MADE WITH FLOUR

Crumb Moisture of Bread	Bread Property	Time of Storage (Hours)			
		20	44	68	140
Higher	Crumb moisture,	44.1	44.0	43.2	40.4
Lower	percent	41.8	41.3	40.3	37.0
Higher	Compressibility,	95	54	46	20
Lower	mm. × 10	73	36	31	15
Higher	Panel judgment	5.03	4.50	4.03	2.81
Lower	of freshness[a]	4.53	4.00	3.32	2.22
Higher	Variance in	0.79	0.69	0.60	0.76
Lower	panel judgment	0.89	0.69	0.73	0.35
	Significance of freshness differences[b]	*	*	***	**

[a] Average of two replications. Same scale as in Table I.
[b] Same symbols as in Table I.

of the test the bread of higher crumb moisture was judged significantly fresher than that of lower moisture.

Tailings starch had little direct effect on the staling characteristics of bread made of flour fractions, when the moisture contents of the

breads were the same. In one case staling tests were made over a 6-day storage period, to compare bread made from gluten and starch with bread made from gluten and a mixture of 90% starch and 10% tailings, at the same crumb moisture. Throughout the test the bread which contained tailings starch was judged slightly fresher. Results of another series of tests are given in Table III. In this case one of the breads, No. 4, was made from gluten and a mixture of 90% starch and 10% tailings. The other bread, No. 5, was made from gluten and a mixture of 80% starch and 20% tailings. Crumb moistures were the same after equal storage periods. In this experiment the bread which contained more tailings fraction was usually judged fresher, but the differences were not significant, statistically.

TABLE III

EFFECT OF TAILINGS FRACTION ON THE FRESHNESS OF BREAD

Bread No.	Bread Property	Time of Storage (Hours)				
		2	20	68	116	140
4[a]	Crumb moisture,	42.6	...	...	...	37.0
5[a]	percent	42.2	...	...	...	37.2
4	Compressibility	...	94	54	29	28
5	mm. × 10	...	107	66	42	26
4	Panel judgment of	...	5.56	4.44	3.50	2.88
5	freshness[b]	...	5.94	4.94	3.44	3.00
4	Variance in	...	0.25	0.87	0.75	0.98
5	panel judgment	...	0.07	0.43	1.25	1.00
	Significance of freshness difference[c]	...	*	none	none	none

[a] Bread No. 4 was made with gluten and a mixture of 90% starch and 10% tailings. No. 5 was made with gluten and a mixture of 80% starch and 20% tailings.
[b] Same scale as in Table I.
[c] Same symbols as in Table I.

Discussion

Bechtel, Meisner, and Bradley (3) showed that when the crust was removed from fresh bread, and the loaf of crumb was stored in a manner to prevent loss of moisture, this crumb staled much less rapidly in the period from 44 hours to 140 hours than did the crumb of intact loaves of bread which were stored in the same manner. During the storage of the intact loaves there was a continuous migration of moisture from crumb to crust. After storage for 68 hours the crumb of the intact loaves had 2% less moisture than when fresh, while after 140 hours it had 4% less moisture. In explanation of the more

rapid staling of the crumb of the intact loaves it was suggested that this might be caused by loss of crumb moisture to the crust, by an undesirable flavor imparted to the crumb by the crust, or by a combination of these processes.

Data obtained in the present experiments and given in Tables I and II show that a difference of 2% in crumb moisture is sufficient to cause a significant difference in the human judgment of the freshness of bread, throughout the period studied. Data in Table I show also that a greater difference in crumb moisture, amounting to almost 4% in the comparison of breads 1 and 3, caused a greater difference in the judgment of freshness of the bread. These findings indicate that migration of moisture from crumb to crust of bread during storage can account for an important part of the staling changes which occur in the period from 20 hours to 140 hours after bread is baked.

The effect of tailings starch on the staling of bread was negligible, when the breads compared had equal crumb moisture, as in the case of breads 4 and 5 of Table III. This does not mean that the tailings starch fraction is without significance in the staling of bread made of flour. As stated above, tailings has a much greater moisture-sorbing capacity than starch. The maximum loaf moisture of bread made with gluten and starch was 32.5% to 33.5% with a crumb moisture of approximately 41% when 2 hours old. Bread made of white flour has a normal loaf moisture of 37% to 38%, with a crumb moisture of approximately 44% when 2 hours old.

Thus the tailings fraction in flour, with its high moisture-sorbing capacity, permits making bread of higher moisture content. The data given here show that such bread is regarded as fresher than similar bread of lower moisture content, after equal storage periods.

Literature Cited

1. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. I. Fractionation of flour and preparation of bread. Cereal Chem. **31:** 163–170 (1954).
2. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. II. Selection of the sensory test panel. Cereal Chem. **31:** 171–175 (1954).
3. Bechtel, W. G., Meisner, D. F., and Bradley, W. B. Effect of the crust on the staling of bread. Cereal Chem. **30:** 160–168 (1953).
4. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives (3rd ed.), by J. A. Radley; Vol. II, Chap. 10. Chapman and Hall: London (1953).
5. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. **16:** 780–792 (1939).

STALING STUDIES OF BREAD MADE WITH FLOUR FRACTIONS.

IV. EFFECT OF GLUTEN AND WHEAT STARCH[1]

W. G. Bechtel and D. F. Meisner[2]

ABSTRACT

Breads made from gluten and starch as the only flour constituents were made with protein content from 10.8% to 17.2% of the gluten and starch mixture. During the first 3 days of storage the breads staled at the same rate. Later in the storage period differences in staling rate developed. As the protein content of the "flour" was increased the breads staled less rapidly.

These results appear to indicate that changes in starch cause the staling which occurs early in the storage period, and that gluten affects staling properties of bread after longer storage periods.

The purpose of these studies was to obtain direct evidence of the effect of gluten and starch on the staling of bread. Breads, having gluten and starch as the only flour constituents, were produced from "flours" containing from 8.5% to 26% protein (14% moisture basis). Although the loaf characteristics of all such breads were reasonably good, only those made of "flour" with protein between 10.5% and 17.5% resembled baker's white bread closely in volume, crumb characteristics, and flavor (5).

Three breads of different gluten and starch composition, within the gluten range found to be entirely satisfactory, were used in these studies. Sensory tests of freshness, and laboratory tests of compressibility, crumbliness, and swelling power, were made at 24-hour intervals throughout the 6-day period.

Materials and Methods

Flour Fractions. Gluten and wheat starch were prepared by methods described previously (5).

Bread. Three breads were prepared in which starch and gluten were used in amounts calculated to give "flours" containing 10.8%, 12.9%, and 17.2% protein (14% moisture basis). The baking procedure has been described in general terms (5). For the breads used in these studies the quantities not specified in the general procedure were:

[1] Manuscript received November 4, 1953. The study on which this article is based was made by the American Institute of Baking under contract with the U. S. Department of Agriculture and under the authority of the Research and Marketing Act.

[2] Present address: Omar Incorporated, Omaha, Nebraska.

Protein in "flour"	*10.8%*	*12.9%*	*17.2%*
Sponge			
Gluten (a)	142.5 g.	171 g.	228 g.
Starch (b)	267.0 g.	255 g.	232 g.
Water (c)	127.0 ml.	92 ml.	57 ml.
Dough			
Gluten (a)	142.5 g.	171 g.	228 g.
Starch (b)	267.0 g.	255 g.	232 g.
Water (c)	60.0 ml.	60 ml.	25 ml.

(a) Gluten was stored frozen without being dried. Amounts given above are for gluten of 65.0% water and 70.1% protein, dry basis. Different batches varied within ±2% in moisture and protein. These amounts of gluten and starch yield 650 g. "flour" of 14% moisture, sufficient for two 1-lb. loaves of bread.
(b) Calculated to 14% moisture.
(c) A considerable proportion of the required water was contained in the gluten. The amounts of water were decreased with increased gluten content in order that the three breads would have the same moisture content.

Bread Storage. As soon as the loaves were sufficiently cool, about 1 hour after leaving the oven, they were wrapped in waxed paper which was then heat-sealed. They were stored in a cabinet at 75°F. until immediately before testing.

Sensory Tests. These were made by the rating scale method described previously (6) using the panel of 20 trained members selected on the basis of superior ability to discriminate between bread samples of different ages, and of reliability in scoring replicate samples. Of the 18 different samples required to test three breads each day during a 6-day storage period, all but three were given at least twice, and six were given three times. Intervals between repetitions were from 1 to 4 weeks. Results in this paper are averages of the replicate tests. Dispersion of individual judgments for each sample is shown in terms of the variance of the tests.

Laboratory Tests. Bread moisture was determined by the A.A.C.C. method (1). Crumb compressibility and crumbliness were tested by methods described by Bechtel, Meisner, and Bradley (8). Crumb swelling power measurements were made by the procedure of Schoch and French (11). Laboratory tests were made on one loaf of each batch while the other was given to the panel for judgment, thus insuring that panel and laboratory evaluations were made on loaves of identical properties.

Results

Data obtained from sensory tests, together with moisture analyses, and tests of crumb compressibility, crumbliness, and swelling power are given in Table I. In the judgment of the panel there were no appreciable differences in the freshness of the breads when they

TABLE I

EFFECT OF PROPORTION OF FLOUR PROTEIN ON SENSORY JUDGMENT OF FRESHNESS, AND ON CERTAIN PHYSICAL PROPERTIES OF BREAD DURING A 6-DAY STORAGE PERIOD

Protein in Flour	Bread Property	Storage Time (Hours)						
		2	20	44	68	92	116	140
%								
10.8	Crumb	40.9	...	...	...	...	...	36.4
12.9	moisture,	40.6	...	...	...	...	...	36.1
17.2	percent[a]	40.8	...	...	...	...	...	36.6
10.8	Panel judg-	...	5.50(1)	4.20(1)	3.95(2)	3.40(2)	3.12(2)	2.55(2)
12.9	ment of	...	5.57(2)	4.95(3)	3.92(3)	4.15(3)	3.77(2)	3.27(2)
17.2	freshness[b]	...	5.60(2)	4.53(2)	4.18(3)	3.90(1)	4.28(2)	4.07(2)
10.8	Variance in	...	0.35	0.56	0.80	0.94	0.96	0.70
12.9	panel	...	0.40	0.62	0.68	0.89	0.92	1.00
17.2	judgment	...	0.24	1.00	0.69	0.69	0.61	0.77
10.8 & 12.9	Significance of	...	none	***	none	***	**	***
12.9 & 17.2	differences in	...	none	*	none	none	**	***
10.8 & 17.2	freshness	...	none	none	none	*	***	***
	judgment[c]							
10.8	Crumb	157	86	46	36	28	16	14
12.9	compressi-	180	104	52	37	32	22	16
17.2	bility	123	88	38	38	38	38	36
10.8		...	8.6	21.4	22.2	19.3	19.2	20.4
12.9	Crumbliness	...	4.4	10.6	20.7	17.2	12.0	13.8
17.2		...	0.8	3.4	4.2	4.4	4.5	4.4
10.8	Crumb	3.60	2.92	2.66	2.53	2.68	2.55	2.44
12.9	swelling	3.65	3.00	2.66	2.49	2.54	2.64	2.59
17.2	power	3.66	3.08	2.78	2.66	2.71	2.64	2.62

[a] Loaf moisture 33±0.5%.
[b] Rating scale: 6, very fresh; 5, fresh; 4, slightly fresh; 3, slightly stale; 2, stale; 1, very stale. Number of replications given in parentheses.
[c] * 5% level; ** 1% level; *** 0.1% level.

were 20 hours old. On succeeding days the breads were found to stale and, while there were some variations from day to day through 92 hours, generally the order of increasing freshness corresponded with the increase in flour-protein content. At 116 and 140 hours this pattern emerged clearly. At 116 hours, for example, the judgments of freshness of the breads made of "flours" containing 10.8%, 12.9%, and 17.2% protein were 3.12, 3.77, and 4.28, respectively, while at 140 hours they were 2.55, 3.27, and 4.07.

It may be observed that the loaf moistures of the breads were approximately the same when 2 hours old, and that there was no appreciable change in 140 hours. Crumb moistures were also in good agreement at the same times. The decrease in crumb moisture in the period from 2 hours to 140 hours was due to migration of moisture from crumb to crust (9). With crumb and loaf moistures in such close agreement, differences in staling rate cannot be attributed to differences in moisture. They must be due to the differences in the proportions of starch and gluten which made up the "flour."

The significance of the differences in freshness, the number of repetitions of each test, and the variances, are given in Table I. Differences

in freshness judgment were generally not significant in tests of bread until after 68 hours' storage. At 116 hours all differences were at or below the 1% level of significance, while at 140 hours all were well below the 0.1% level.

In agreement with results reported in previous papers dealing with the staling process (4, 8), there appears to be no direct relationship between the panel judgment and the physical laboratory tests. For example, breads made with "flours" containing 10.8% and 12.9% protein had about the same compressibility at the same age, in tests from 44 hours to 140 hours, yet from 92 hours to 140 hours the panel reported a great difference in freshness. At 20 hours, when the panel considered the breads equivalent in freshness, the bread of "flour" containing 12.9% protein was appreciably softer. Bread of highest flour protein was much less crumbly than the others of equal age, while that of lowest protein was most crumbly. Yet they were judged by the panel to be of about equivalent freshness from 20 hours to 68 hours. The panel judged the breads made with "flours" containing 10.8% and 12.9% protein to stale by a considerable amount in the period from 68 hours to 140 hours, although there was no consistent change in their crumbliness during that period. Crumb swelling power was about the same for breads of equal age, and changed only a small amount after 44 hours.

Discussion

The physical structure of bread crumb is of great importance in the attempt to explain the staling process. Katz (10) and Baker (3) have shown that the crumb structure of bread is that of a continuous film of gluten which completely surrounds the individual, gelatinized starch granules. Within the gluten the swollen starch granules are distorted, and are oriented so that the longest dimension is approxi-

Gluten and Starch Content of Flour Components in Breads

	%	%	%
Protein in "flour" of bread	10.8	12.9	17.2
	g.	g.	g.
Gluten, wet, per 650 g. "flour" at 14% moisture[a]	285	342	456
Flour protein, dry, in the gluten	70	84	112
Starch, dry, in the gluten[b]	30	36	48
Starch added, dry basis	460	440	400
Total starch, dry basis	490	476	448

[a] Flour for two 1-lb. loaves.
[b] It has been assumed that the dry gluten is composed entirely of protein and starch. Correction for the small amount of fat and ash would not materially alter the results.

mately parallel to the longitudinal axis of the gluten strand. The vast number of such swollen, oriented starch granules in the gluten gives rigidity to the crumb structure of bread.

There was a relatively small change in the amount of starch in the three breads made from "flour" consisting only of gluten and starch, as shown in the following table. "Flours" of 12.9% and 17.2% protein contained 97.1% and 91.4% as much starch, respectively, as did that of "flour" of 10.8% protein. The proportion of gluten varied by a much larger amount. "Flours" of 12.9% and 17.2% protein contained, respectively, 120% and 160% as much gluten as did that of 10.8% protein. From this it appears probable that similarities in staling changes of the three breads were due to changes in starch, while differences in staling were due to gluten.

In these experiments and in studies of the effect of the crust on the staling of bread (8), the different breads staled at the same rate during the first 2 to 3 days of storage. Gels of cereal starches increase rapidly in rigidity during the first 18 hours at room temperature. The increase continues at measurable, though diminishing, rate for at least 48 hours. The swollen granules of such a starch gel are closely packed, and the properties of the gel reflect the growth of a rigid structure within the granules and in the amylose dissolved from them. This period of rapid increase in starch granule rigidity corresponds to that of greatest decrease in crumb compressibility of bread (4, 8; Table I). It seems likely, therefore, that increase in rigidity of the starch granules of bread is the cause of the rapid firming of the crumb which occurs during the first 2 days of storage. In this period the increase in crumb firmness appears to be one of the most important factors in estimating bread freshness by sensory methods.

After the third day of storage, the breads in these experiments showed consistent differences in staling rate (Table I). Breads staled less rapidly from the third to the sixth day of storage as the proportion of gluten to starch was increased. During this period staling changes cannot be ascribed to a further increase in crumb rigidity, nor to changes in any of the commonly measured physical properties (7, 8). It was observed, after storage periods of 3 days or more, that the breads differed considerably in crumb texture. As the gluten content of the "flour" decreased, the crumb became increasingly harsh. At the end of 140 hours, for example, the crumb of bread made of "flour" containing 10.8% protein was very harsh, while that of "flour" containing 17.2% protein remained smooth and velvety to the touch. A similar difference had been observed in comparing the crumb of decrusted and intact bread (8) after storage for several days. Crumb of

decrusted bread, which remained at constant moisture and which was judged to be relatively fresh after 6 days, was less harsh than that of the intact loaves which lost moisture to the crust and staled rapidly.

Since gluten is the continuous phase in bread, crumb texture appears to be a property of gluten, and of the proportion of gluten to starch. In bread of normal moisture, one would expect that with an increase in proportion of gluten to starch the crumb would have greater resilience, and hence a smoother texture. Gluten becomes harsh as it dries. The gluten of crumb may become dry by exposure to air, by loss of moisture to the crust and, as indicated by the work of Bachrach and Briggs (2), by a small loss of moisture to the starch.

Bachrach and Briggs showed that the starch in bread increased somewhat in moisture-sorbing capacity on aging. From this, the moisture which migrates from crumb to crust during the storage of bread would come largely from the gluten. It follows that as the proportion of gluten is increased the migration of a given amount of moisture to the crust would cause less drying per unit weight of gluten.

It thus appears that bread staling is caused, to a large extent, by two separate processes. During the first 2 or 3 days of storage the most important staling change is firming of the crumb caused by increased rigidity of the starch gel. After longer storage increased crumb harshness, caused by loss of moisture from gluten, is of major importance.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.), pp. 155–156. The Association: St. Paul, Minn. (1947).
2. Bachrach, H. L., and Briggs, D. R. Studies on bread staling. II. Water relationships during staling of bread crumb and the retrogradation of starch. Cereal Chem. **24**: 492–506 (1947).
3. Baker, J. C. The structure of the gas cell in bread dough. Cereal Chem. **18**: 34–41 (1941).
4. Bechtel, W. G., and Meisner, D. F. Present status of the theory of bread staling. Food Technology **5**: 503–505 (1951).
5. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. I. Fractionation of flour and preparation of bread. Cereal Chem. **31**: 163–170 (1954).
6. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. II. Selection of the sensory test panel. Cereal Chem. **31**: 171–175 (1954).
7. Bechtel, W. G., and Meisner, D. F. Staling studies of bread made with flour fractions. III. Effect of crumb moisture, and of tailings starch. Cereal Chem. **31**: 176–181 (1954).
8. Bechtel, W. G., Meisner, D. F., and Bradley, W. B. The effect of the crust on the staling of bread. Cereal Chem. **30**: 160–168 (1953).
9. Bradley, W. B., and Thompson, J. B. The effect of crust on changes in crumbliness and compressibility of bread crumb during staling. Cereal Chem. **27**: 331–335 (1950).
10. Katz, J. R. Staling of bread and means of retarding it. Bakers' Weekly **82(1)**: 40, 52 (1934).
11. Schoch, T. J., and French, D. Studies on bread staling. I. The role of starch. Cereal Chem. **24**: 231–249 (1947).

STUDIES ON BREAD STALING. V. EFFECT OF FLOUR FRACTIONS AND VARIOUS STARCHES ON THE FIRMING OF BREAD CRUMB[1]

NEVILLE PRENTICE,[2] L. S. CUENDET,[3] AND W. F. GEDDES[4]

ABSTRACT

The influence of flour fractions from hard red spring and soft red winter wheat flours, of rye flour water-solubles, and of several natural and modified starches upon the firming of bread crumb was studied. Gluten, starch, starch tailings, and water-solubles separated from the flours were combined to yield synthetic flours designed to reveal the effect of individual constituents on the crumb firmness of bread after various storage times from 4 to 69 hours at 25°C. In the experiments involving different natural and modified starches, which showed wide variations in transition temperature (the temperature at which 20% slurries showed an increase in viscosity when heated in the Brabender Amylograph), the starches replaced one-third of the wheat starch.

Increasing the protein content of the synthetic flours but maintaining a constant ratio of gluten to water-solubles increased absorption and loaf volume but decreased the average crumb firmness and crumb firming rate. These findings were confirmed with bread baked from soft wheat flour which had been enriched with gluten to 13.5 and 16.5% protein.

Substituting soft flour starch or gluten for hard flour starch or gluten increased average crumb firmness but did not affect the firming rate.

Starch tailings had no effect on loaf volume or crumb firming rate but this fraction from both hard and soft flour decreased the average crumb firmness.

Hard and soft flour water-solubles and especially those of rye flour increased absorption and loaf volume and decreased both average crumb firmness and crumb firming rate.

Substitution of any of the starches except rye for one-third of the wheat starch decreased loaf volume.

The rates of crumb firming for bread containing cassava, rye, and oat starch, which had lower transition temperatures than wheat starch, did not differ from that of bread made from the control. Oat starch, but not rye or cassava starches, decreased average crumb firmness. The average crumb firmness and firming rate of breads containing corn, rice, and especially waxy corn and waxy sorghum starch, which have higher transition temperatures than wheat starch, were greater than bread from the control flour. All the modified starches caused an increase in average crumb firmness; with the exception of a cross-bonded ether derivative of corn starch in which swelling was markedly inhibited, they also increased the crumb firming rate.

Although organoleptic tests by a panel of experts are the most valid means of judging the staleness of bread, the development of a relatively rigid crumb structure is one of the most obvious of several pro-

[1] Manuscript received February 18, 1954. Paper No. 3119, Scientific Journal Series, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois, and has been assigned number 473 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

The data in this paper are to be included in a thesis to be submitted to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for the M.S. degree.

[2] Research Assistant, Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

[3] Assistant Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

[4] Professor of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

gressive changes which occur in bread during aging (7, 8, 9,16). The softness of bread appears to be the criterion used by the consumer at the time of purchase to estimate freshness and it is not surprising that measurements of the compressibility of bread crumb have been extensively employed as an index of its consumer acceptability (9, 10).

It is generally believed by baking technologists that bread from strong flours is of better keeping quality than that made from weak flours (1, 18). However, there are few experimental data available on the effect of variations in the quantity and quality of flour proteins and of other constituents upon the rate of crumb firming.

Steller and Bailey (27) found an inverse relation between the protein content of four flours and the rates at which the softness of breads made from them decreased upon storage. However, the keeping quality of the bread, as measured by crumb softness, did not appear to be a linear function of protein content, indicating that protein characteristics and other factors inherent in the flour are operative. That protein quality may be important was shown by the more rapid decrease in crumb softness of bread made from a soft wheat flour (protein content, 10.1%) than of that from a semi-hard wheat flour (protein content, 9.6%).

Of the flour constituents, the role of starch in crumb firming has received the most attention. Extensive researches have shown that changes in the starch fraction are of great importance in the aging of bread (9, 10, 26). Recent work indicates that it is mainly the amylopectin and not the amylose fraction that contributes to the firming of bread crumb. Noznick, Merritt, and Geddes (21) tested the compressibility and swelling power of bread crumb from micro loaves baked from mixtures of gluten and starch. Replacing some of the wheat starch by waxy maize and sorghum starch did not decrease crumb firming; crumb swelling power, however, was increased by the presence of these starches. Schoch and French (26) concluded, on the basis of potentiometric iodine titrations, that the "soluble starch" leached from bread at 30°C. with water consists mainly of the branched amylopectin fraction and that the amylose component of starch is nearly completely retrograded during baking. The quantity of amylopectin extractable decreased with the aging of the bread and they regarded the heat-reversible aggregation of amylopectin as a prime factor in the bread-staling process. Gilles (17), however, has shown that the so-called soluble-starch fraction from bread crumb contains pentosans, the quantities of which increase with a decrease in the amount of this fraction as the bread ages. Also the wash water from wheat starch contained a pentosan fraction which gave a blue

color with iodine. There is some doubt concerning the reliability of the iodine sorption technique for the determination of amylose in starch.

Several workers have drawn attention to the importance in bread-making of the water-soluble components of wheat flour and of the impure starchy material which is obtained as an upper layer in recovering starch from the wash water during the preparation of gluten. This mixture, which contains protein, pentosan, and inorganic constituents in addition to starch (20, 22), has been variously designated as the "amylodextrin fraction" (24), the tailings fraction (20), and "squeegee" starch (14). Sandstedt *et al.* (24) found that synthetic flours composed of gluten, starch, and "amylodextrin" produced bread which compared favorably with that made from the flours from which the fractions had been prepared; the water-soluble fraction did not appear to play a significant role in the baking process. Finney, however, was unable to obtain a good loaf from Thatcher gluten and starch without the water-solubles (15). Pence, Elder, and Mecham found that the water-soluble fraction of flour was necessary for maximum performance of glutens from several wheat varieties (23). There have been no investigations concerning the effect of the water-soluble components on the changes which occur during the storage of bread.

Wheat flours vary so widely in protein quantity and in protein quality, as well as in other characteristics, that they are not suitable experimental materials for studying individually the effects of various flour constituents upon the firmness of bread. The use of synthetic flours, however, affords a means of studying the effects of each flour constituent independently of variations in the properties of the other components. The present investigation was undertaken to determine the effect of gluten, starch, tailings, and water-soluble fractions from a strong and a weak wheat flour and of several starches having different gelatinization characteristics upon the firming of bread crumb made from suitable mixtures of these substances. To secure a wide range in starch properties, several commercially available corn starches which had been modified by various chemical treatments were employed in addition to cassava, wheat, and other natural cereal starches. The effect of water-solubles obtained from rye flour was also investigated.

Materials and Methods

Preparation of Flour Fractions. Gluten and other fractions were prepared from two types of wheat flour, neither of which had received any bleaching or maturing treatment. These were two hard red spring

wheat baker's patent flours and a soft red winter wheat flour which is not normally used for breadmaking. Successive kilogram quantities of each were made into a dough with distilled water at about 4°C., and the gluten was recovered by washing with further quantities of distilled water at this temperature in order to minimize enzymatic action. Centrifuging the starch milk yielded the water-soluble fraction (centrifugate), the starch and "tailings." The tailings formed a layer on top of the more dense starch in the centrifuge bottles and was removed by means of a spatula. All fractions were frozen and dried from the frozen state. The gluten, starch, and tailings were pulverized but no attempt was made to reduce the particle size of the water-soluble fraction because of its hygroscopic nature. The corresponding fractions obtained from the various lots were composited and thoroughly mixed in a MacLellan batch mixer. Water-solubles were obtained from rye flour by preparing a slurry in distilled water (about 4°C.), centrifuging, and drying the frozen centrifugate. The protein contents of the original flours and the various fractions were determined in the usual manner (2), and the data together with the yields of the different hard flour fractions are recorded in Table I. Because

TABLE I

YIELDS AND PROTEIN CONTENTS OF FLOURS AND FLOUR FRACTIONS[a]

Fraction	Hard Red Spring Wheat				Soft Red Winter Wheat	Rye
	Yield		Protein			
	No. 1	No. 2	No. 1	No. 2	Protein	Protein
	%	%	%	%	%	%
Original flour	...	..	12.1	12.1	8.1	...
Gluten	15.3	13.3	69.7	73.8	68.9	...
Starch	66.4	69.7	0.4	0.4	0.7	...
Tailings	12.4	10.7	1.5	2.3	4.0	...
Water-solubles	5.9	6.3	15.1	27.5	28.4	25.4

[a] All the analytical data are expressed on a 14.0% moisture basis. Two lots of hard red spring wheat flour were fractionated; No. 1 contained 0.43% ash and No. 2, 0.47% ash. The ash content of the soft red winter wheat flour was 0.46%.

of the difficulties involved in washing gluten from the soft wheat flour, no attempt was made to fractionate it on a quantitative basis.

Preparation of Starches. In addition to the wheat starch obtained by fractionating the wheat flours, other cereal starches and cassava starch were prepared or obtained. Also, several commercially available modified starches were selected to provide a wide range in swelling properties.

Rice and rye starches were prepared by suspending the appropriate

flours in distilled water at about 4°C. and centrifuging. The protein and bran layer on top of the starch was discarded and the moist starch dried at room temperature with a fan.

Sulfur dioxide had been used in the preparation of many of the starches obtained from other sources; since this reducing agent influences the baking properties of wheat flour, it was necessary to determine the amount present and, in some cases, to adjust the level by treatment with hydrogen peroxide. The sulfur dioxide content of the starches was determined by digesting the oxygen-free starch with 0.6 *N* hydrochloric acid solution under carbon dioxide and receiving the sulfur dioxide released in a 3% hydrogen peroxide solution (4). The sulfuric acid produced was then titrated with standard sodium hydroxide solution using bromophenol blue indicator. No attempt was made to decrease the sulfur dioxide content of starches which contained less than 1 mg. % since this was found to require a large excess of hydrogen peroxide which affected the physical properties of the starch.[5] Where necessary to lower the sulfur dioxide content, slurries of the starch were treated with an amount of hydrogen peroxide slightly in excess of that required to react with the sulfur dioxide present. The starch was recovered by centrifugation and dried at room temperature with a fan.

To secure a measure of the swelling properties of the various starches, a slurry of 100 g. starch (14.0% moisture basis) in 400 g. distilled water was gelatinized in the Brabender Amylograph. The temperature at which the viscosity began to increase was recorded as the transition temperature, as described by Anker and Geddes (3). It was necessary to use 20% slurries since many of the modified starches failed to show any increase in viscosity at lower concentrations. Maximum viscosities for the starch slurries could not be determined with the amylograph at constant starch concentration, since at any one concentration the viscosity of some slurries did not increase while others exceeded the capacity of the instrument.

The nature, source, and analytical data for the various starches are given in Table II.

Preparation of Synthetic Flours. To determine the effect on crumb firming of the various flour fractions and starches listed in Tables I and II, 29 synthetic flours representing various combinations containing gluten were prepared. In one series, the hard flour fractions (No. 2, Table I) were combined to yield five protein levels from 10.1 to 16.5%. The quantity of starch tailings was held constant at 10.8%

[5] Personal communication from T. J. Schoch, Corn Products Refining Company, Argo, Illinois.

TABLE II

NATIVE AND MODIFIED STARCHES USED IN PREPARING SYNTHETIC FLOURS

Name	Source	Description	Protein[a]	Sulfur Dioxide[a]	Transition Temperature
			%	*mg.* %	°C.
1. Cassava	American Maize Products Co., Roby, Indiana	Native	0.0	0.2	49.0
2. Rye	Prepared in this laboratory from rye flour	Native	0.5	0.0	50.5
3. Oat	Northern Utilization Research Branch, U.S.D.A., Peoria, Ill.	Native	0.0	0.0	53.5
4. Wheat	Prepared in this laboratory from wheat flour	Native	0.4	0.0	55.0
5. Waxy corn	Northern Utilization Research Branch	Native	0.0	0.0	58.7
6. Corn	American Maize Products Co.	Native	0.3	0.5	61.0
7. Rice	Prepared in this laboratory from rice flour	Native	3.3	0.0	61.0
8. Waxy sorghum	Northern Utilization Research Branch	Native	0.5	0.6	62.5
9. "Nu Film"**	National Starch Products Co., New York, N. Y.	Sodium salt of an ungelatinized low-substituted acid ester derivative of corn starch containing COOH and SO_3H groups.	0.0	0.6	32.5
10. "G-2"**	National Starch Products Co., New York, N. Y.	Corn starch derivative of succinic acid having high water-retaining properties.	0.2	0.2	50.5
11. "Hercules 55"**	Corn Products Refining Co.	Thin-boiling, oxidized corn starch prepared by cold alkaline hypochlorite treatment.	0.0	0.2	55.0
12. "Penford gum 280"**	Penick and Ford Co., Cedar Rapids, Iowa	Hydroxyethyl ether of a thin-boiling,acid-modified corn starch.	0.2	0.2	56.5
13. "Vulca 30"**	National Starch Products Co.	Cross-bonded ether derivative of corn starch which resists swelling.	0.2	0.2	60.2
14. "Vulca 100"**	National Starch Products Co.	Similar to Vulca 30 but has a higher degree of cross-bonding.	0.3	0.2	*
15. "Amioca 85"**	American Maize Products Co.	Thin-boiling, acid-modified waxy maize starch.	0.3	0.6	*

[a] Data are expressed on a 14.0% moisture basis, protein = N × 5.7.
* No viscosity increase at or below 93°C.
** Copyrighted trade names of modified starches marketed by the indicated manufacturer.

and the changes in protein content were obtained by varying the quantities of gluten, water-solubles, and starch but at the same time maintaining a constant ratio of gluten to water-solubles. The composition of these mixtures is given in Table III.

TABLE III

COMPOSITION OF SYNTHETIC FLOURS OF VARYING PROTEIN CONTENT[a]
(Prepared with fractions from hard wheat flour No. 2)

Flour No.	Protein Content[b]	Gluten	Starch	Tailings	Water-Solubles
	%	%	%	%	%
1	10.5	11.5	72.3	10.8	5.4
2	12.1	13.4	69.6	10.8	6.3
3	13.5	15.0	67.2	10.8	7.0
4	15.0	16.7	64.6	10.8	7.9
5	16.5	18.5	62.1	10.8	8.7

[a] Data are expressed on a 14.0% moisture basis.
[b] Computed from the quantities of the various fractions employed and their respective protein contents as given in Table I.

In a second series, seven synthetic flours (Nos. 6–12) of the same protein content (13.5%) were prepared using different combinations of the respective fractions from the hard and soft flour, as well as substituting the water-solubles from rye flour for those from the wheat flours. The composition of these mixtures is given in Table IV.

TABLE IV

COMPOSITION OF SYNTHETIC FLOURS OF 13.5% PROTEIN CONTAINING DIFFERENT COMBINATIONS OF FRACTIONS FROM HARD AND SOFT WHEAT FLOURS AND RYE WATER-SOLUBLES

Flour No.	Description	Composition[a]			
		Gluten	Starch	Tailings	Water-Solubles
		%	%	%	%
6	Tailings omitted	15.2	77.7	0.0	7.0
7	Tailings from soft flour	15.0	72.5	5.5	7.0
8	Starch from soft flour	14.7	67.4	10.8	7.0
9	Water-solubles from soft flour	15.0	67.4	10.8	6.8
10	Gluten from soft flour	16.0	66.1	10.8	7.0
11	Water-solubles decreased by one-half	16.3	69.4	10.8	3.5
12	Rye flour water-solubles	15.0	66.6	10.8	7.6

[a] Unless otherwise stated, the fractions employed in making the mixtures were obtained from hard flour No. 2. Data are expressed on a 14% moisture basis.

As a further test of the effect of protein quality on crumb firming, two flours (Nos. 13 and 14) were prepared by adding gluten from hard flour No. 2 to the soft red winter wheat flour (protein, 8.1%) to bring the protein content to 13.5 and 16.5% respectively.

Since the baking behavior of the modified starches and of several of the native starches listed in Table II was unknown, a preliminary series of synthetic flours was prepared in which one-third and one-half of the wheat starch obtained in fractionating hard flour No. 1 was replaced by each starch. Micro baking tests revealed that several of the mixtures, particularly those containing the waxy starches, "Nu Film," "G-2," "Vulca 100," and "Amioca 85," would not yield a satisfactory loaf of bread when the starches replaced one-half of the wheat starch. Consequently, all starches were used to replace only one-third of the wheat starch in preparing mixtures which, in other respects, corresponded in composition to hard flour No. 1 of 12.1% protein content (flours 15 to 29). The protein contents of these starches were disregarded since the quantity and quality of the nitrogenous constituents would not be expected to influence the baking properties appreciably.

Baking Procedure and Measurement of Crumb Firmness. To prepare bread for the crumb firming tests, 800 g. of flour were used in a baking formula containing 5.0% sucrose, 1.0% sodium chloride, 3.0% yeast, and 0.66% calcium propionate based on the weight of flour. Distilled water was added to give the desired consistency and the doughs were mixed in a Hobart mixer equipped with a McDuffee bowl for 3 minutes at first speed and 1 minute at second speed followed by 1 minute at first speed. Each dough was divided into five 250-g. portions and fermented at 30°C. Doughs from flours 1 to 14 were fermented for 120 minutes and punched after 95 minutes, while those from flours 15 to 29 were fermented 90 minutes and punched after 60 minutes. The preliminary baking trials showed that these fermentation times gave the best results. All doughs were proofed for 55 minutes at 30°C. and baked for 22 minutes at 235 ± 5°C. After 1 hour the loaves were weighed and the volumes determined by seed displacement after which they were stored in sealed containers at 25±1°C. for crumb firmness measurements. At the second storage period the loaves were scored for crumb grain using a scoring range of 0 to 10. As it was not possible to bake all flours on the same day, they were divided into several groups, each with an appropriate control flour.

Crumb firmness was measured with a Baker Compressimeter (2) at storage periods of 4, 20, 31, 44, and 69 hours after baking. At each interval one loaf was sliced in a miter box into eight slices about 1.5

cm. thick. A section 6 × 6 cm. was cut from the center of each slice and the weight required to compress the bread a given distance (1.0 to 4.0 mm.) was determined. (Plunger diameter was 2.54 cm.) By this procedure, a series of eight replicate measurements was made for each staling period.

Statistical Treatment of Data. The different synthetic flours gave bread of widely varying loaf volumes, and differences in initial crumb firmness would be expected owing to variations in the mass of crumb under the plunger of the compressimeter. The question arose whether these effects of crumb mass on the crumb firmness values could be eliminated by correcting the data for the different samples on the basis of the relative loaf volumes of the breads in comparison with a control. An experiment was conducted in which statistical analyses were made using adjusted and unadjusted crumb firmness data for breads baked from the same flour in such a way as to yield different volumes. Correcting the compressimeter data to compensate for differences in loaf volume did not eliminate day-to-day differences nor appreciably decrease the standard error. Accordingly, the experimental data were analyzed without correcting them to a uniform volume basis.

Variance analyses were made to determine whether any particular variation in the composition of a synthetic flour in comparison with a control had a significant influence on the average crumb firmness over 69 hours' storage, as indicated by the F values for "between flours," and on the rate of crumb firming as indicated by F values for the interaction between flours and storage times. The direct comparison of means was made possible by the calculation of the minimum differences required for significance between the means for individual flours and storage times and those required for significance between the means of individual flours over all storage times.

Results and Discussion

Effect of Different Flour Fractions on Loaf Volume and Crumb Firming. The mean loaf volumes and compressimeter data for synthetic flours prepared from the different flour fractions are recorded in Table V. The flours are separated into three groups according to the day on which they were baked. The synthetic flour of 13.5% protein content prepared from the hard spring wheat flour fractions was baked each day to serve as a control. Variance analyses were made of the compressimeter data for the control and each individual flour, and the pertinent results are summarized by the F values for the variances "between flours," and for the interaction of "flours × storage

TABLE V

EFFECT OF VARIOUS FLOUR CONSTITUENTS ON LOAF VOLUME AND CRUMB FIRMNESS AFTER VARIOUS STORAGE TIMES AT 25°C.

Flour No.	Description	Loaf Volume[a]	Mean Load (g. × 10^{-1}) for 2.5 mm. Deformation After Various Storage Times (Hours)						F values		Minimum Significant Difference[b]	
			4	20	31	44	69	All	Between Flours	Flours × Times	1	2
		cc.										
	Group I. Protein Series from Hard Flour Fractions											
1	10.5% protein	918	5.9	14.5	15.0	17.2	21.2	14.8	151.2**	4.8**	1.7	0.8
2	12.1% protein	1052	4.4	9.4	13.4	11.1	16.3	10.9	12.3**	5.6**	1.1	0.6
3	13.5% protein, control	1282	3.9	7.4	10.9	12.3	15.3	10.0				
4	15.0% protein	1399	3.5	6.2	8.2	7.9	9.5	7.1	194.4**	22.8**	0.8	0.3
5	16.5% protein	1536	3.5	4.8	5.9	7.2	7.7	5.8	626.4**	55.1**	0.8	0.3
	Group II. Synthetic Flours Containing 13.5% Protein											
3	Control, all hard flour fractions	1312	3.7	7.1	9.1	11.3	13.9	9.0				
6	Tailings omitted	1322	4.7	8.8	10.6	12.0	15.0	10.2	33.6**	0.7	0.8	0.6
7	Tailings from soft flour	1305	4.6	6.5	8.6	11.6	14.2	9.1	0.1	1.1	1.1	0.6
8	Starch from soft flour	1233	3.4	8.0	10.8	12.6	14.3	9.8	12.1**	2.4	1.1	0.6
9	Water-solubles from soft flour	1257	3.8	7.5	9.9	11.3	13.0	9.1	0.1	1.3	1.1	0.6
	Group III. Synthetic Flours Containing 13.5% Protein and Soft Flours Enriched with Hard Flour Gluten											
3	Control, all fractions from hard flour	1297	3.9	7.1	8.8	11.0	13.8	8.9				
10	Gluten from soft flour	1171	4.8	8.5	9.9	12.8	14.5	10.1	22.0**	0.6	1.1	0.6
11	Water-solubles decreased one-half	1131	6.2	12.0	12.5	14.7	18.0	12.7	228.4**	2.8*	1.1	0.6
12	Water-solubles from rye flour	1405	3.7	6.4	6.4	8.5	9.0	6.8	122.7**	17.0**	0.8	0.3
13	Soft flour plus hard flour gluten; protein 13.5%	1282	4.8	7.8	9.1	11.4	16.9	10.0				
14	Soft flour plus hard flour gluten; protein 16.5%	1430	4.6	7.6	9.2	9.8	12.1	8.7	33.1**	4.7**	1.1	0.6

[a] Mean of five loaves.
[b] Values in column labeled 1 apply to means for individual storage times. Values in column 2 apply to means for each sample over all storage times.
* Denotes significance at 5% level.
** Denotes significance at 1% level.

times." The variances for "between flours" apply to the mean crumb firmness values over all storage times for each individual flour in comparison with the control. The interaction variance is a measure of the relative rates of crumb firming for the control and each respective flour. Significant F values result when the crumb firmness of the two samples changes at different rates during storage.

An increase in the protein content of the flours prepared entirely from the hard wheat flour fractions was accompanied by a progressive increase in loaf volume. The absorption, which is not shown in the table, increased from 58.0% for the flour containing 10.5% protein to 63.0% for the one with 16.5% protein. The average crumb firmness decreased with increasing protein content; the rate at which the crumb became firm upon storage was also slower for the higher protein samples. The importance of protein in increasing the softness of bread crumb and lessening the rate at which it becomes hard on storage is also revealed by the compressimeter data for the breads made from the soft wheat flours which were enriched to 13.5% and 16.5% protein (flours 13 and 14).

The influence of protein quality is shown by the data for flours 3 and 10; these flours are of the same composition but the glutens were obtained from hard and soft wheat flours respectively; flour 10, containing soft wheat gluten, gave bread of lower loaf volume and greater crumb firmness than flour 3 but there was no significant difference in the rate of firming upon storage.

The influence of the starch tailings fraction is shown by the data for flours 3, 6, and 7 in group II which gave very similar volumes. When the tailings were omitted (flour 6) the average crumb firmness was greater than when they were included in preparing the hard flour (No. 3), but the rates of crumb firming were the same for both breads. This fraction is known to contain a relatively high proportion of starch granules which are damaged in milling and they are more readily hydrated than the undamaged granules of the major starch fraction. Omission of the tailings fraction lowered the absorption of the hard flour from 60 to 58% and the effect of the tailings on crumb firmness is probably due to its relatively high hydration capacity. Substituting the tailings from the soft flour for those of the hard flour had no significant influence on loaf volume, crumb firmness, or firming rate.

Soft wheat flour starch yielded bread with a greater average crumb firmness than hard flour starch, but the rate of crumb firming was similar. This indicates that the starch as well as the lower quantity and quality of the wheat flour proteins may contribute to the firmness of

bread crumb normally associated with weak flours.

The effect of the water-solubles of wheat and rye flour is shown by comparing the data for flours 3, 9, 11, and 12 in groups II and III. When the water-solubles of the soft flour were substituted for those of the hard flour, the absorption was 1.0% higher and the loaf volume 55 cc. lower, but there was no significant difference in the average crumb firmness or rate of crumb firming. When only one-half the quantity of water-solubles obtained in fractionating the hard flour was employed, the absorption was increased 2.0%, the loaf volume decreased 181 cc., and the average crumb firmness and rate of firming increased. Replacing the water-solubles of the hard flour with those from rye flour had no influence on absorption, increased the loaf volume 93 cc., and substantially decreased the average crumb firmness and the rate at which the firmness increased upon storage. These results showed that the water-solubles have a rather pronounced effect. Bread was not tested from flours where water-solubles were omitted entirely, because preliminary baking tests showed that the loaf volume of such bread was inferior and the crumb was unsuitable for compressimeter measurements.

Effects of Different Native and Modified Starches on Loaf Volume and Crumb Firmness After Different Storage Times. In this series one-third of the wheat starch was replaced by the various starches under investigation[6]. There was only sufficient of the hard synthetic flour (No. 20) to use as a control for 2 days' bake. The original flour (hard flour No. 1) from which the fractions were obtained was used on the other 2 days required for completion of the baking tests as well as on a day when flour 20 was baked. The bread made from synthetic flour 20 had an appreciably lower loaf volume than that from hard flour 1 from which the fractions were obtained. This was probably caused by oxidative changes during the fractionation procedure since the dough from flour 20 showed overdevelopment. As would be expected, the crumb firmness of the bread from flour 20 was the greater, and in order to correct for day-to-day variations in baking conditions it was necessary to adjust the compressimeter data for the breads made from synthetic flours, on the days when flour 20 was not baked, to a common control basis. Plots of the mean compressimeter data for hard flour 1 baked on the 3 days showed that there was a linear relation between crumb firmness and storage time. Regression equations were computed

[6] Preliminary microbaking tests employing different starches in synthetic flours indicated that very satisfactory bread could be obtained with a protein level of 12.1%. This level was therefore used in the preparation of breads for the compressimeter measurements. The resulting loaf volumes were, however, smaller than had been anticipated. As these experiments were carried out before those involving the various flour fractions, the protein content for the latter was raised to 13.5%.

TABLE VI

EFFECTS OF REPLACING ONE-THIRD OF THE WHEAT STARCH OF SYNTHETIC FLOURS WITH VARIOUS NATIVE AND MODIFIED STARCHES UPON LOAF VOLUME AND CRUMB FIRMNESS

Flour No.	Starch	Transition Temperature	Loaf Volume[a]	Mean Load (g. × 10^{-3}) for 2.5 mm. Deformation after Various Storage Times (Hours)						F Values		Minimum Significant Difference[b]	
				4	20	31	44	69	All	Between Flours	Flours × Times	1	2
		°C.	cc.										
15	Nu Film	32.5	905	7.5	14.1	15.9	21.6	26.0	17.0	80.3**	10.4**	2.0	0.8
16	Cassava	49.0	1060	6.1	12.4	13.2	14.6	18.4	12.9	0.0	0.3	6.5	2.8
17	Rye	50.5	1132	4.9	9.6	16.3	16.4	17.6	13.0	0.1	2.8*	1.4	0.6
18	G-2	50.5	999	6.0	14.2	15.7	17.2	23.0	15.2	49.2**	12.5**	1.4	0.6
19	Oat	53.5	1063	4.5	8.2	13.0	13.0	14.6	10.7	58.6**	0.5	1.4	0.6
20	Wheat, control	55.0	1128	6.7	10.0	14.7	15.8	17.2	12.8				
20	Wheat, control[c]	55.0	1152	5.5	8.9	11.3	11.1	15.5	10.5				
21	Hercules 55	55.0	1119	8.0	14.1	14.2	15.8	23.8	15.2	262.5**	17.5**	1.1	0.6
22	Penford gum 280	56.5	1056	7.9	14.8	17.1	24.1	24.8	17.7	118.3**	9.8**	2.0	0.8
23	Waxy corn	58.7	1080	3.4	13.2	18.9	21.1	26.0	16.5	12.6**	3.7**	4.5	2.0
24	Vulca 30	60.2	1032	6.2	12.7	16.0	18.7	18.6	14.4	120.6**	9.5**	1.7	0.8
25	Corn	61.0	1065	6.7	15.5	20.1	22.9	26.8	18.4	228.7**	18.8**	1.7	0.8
26	Rice	61.0	1055	4.8	11.5	14.0	19.1	24.2	14.7	27.7**	20.8**	1.4	0.6
27	Waxy sorghum[d]	62.5	895	3.5	16.5	28.3				141.2**	105.2**	1.7	0.8
28	Vulca 100	...	978	14.2	15.8	17.3	17.7	22.5	17.5	219.7**	11.1**	1.4	0.6
29	Amioca 85[d]	[e]	998	3.1	13.8	16.7	20.5			136.2**	44.5**	1.4	0.8

[a] Mean of five loaves.
[b] Values in column labeled 1 apply to means for individual storage times.
Values in column 2 apply to means for each sample over all storage times.
[c] Control for flours 21, 24, and 29.
[d] Crumb firmness increased so rapidly that compressimeter measurements for No. 27 could not be made after 31 hours' storage, nor for No. 29 after 44 hours. Data for the control breads over these respective storage periods were used in the statistical analyses. The average crumb firmness values over 31 hours' storage were: control, 10.5; No. 27, 16.1. For 44 hours' storage the values were: control, 9.2; No. 29, 13.5.
[e] No viscosity increase at or below 93°C.
*Denotes significance at 5% level.
**Denotes significance at 1% level.

for the data for each day and the actual load values revised in accordance with these equations. Correction factors were then computed from these revised values in order to bring the compressimeter data for each bread and storage times used on the different days to a common basis. Actually, the compressimeter values for hard flour 1 were quite consistent for the different days and the correction factors were therefore small. The adjusted data are recorded in Table VI together with the F values obtained from variance analyses.

The substitution of any of the starches except rye for wheat starch decreased the loaf volume of the bread. The rye starch, however, caused an open coarse crumb. Cassava, rice, and corn starches also produced bread with a coarse crumb. The waxy starches (waxy corn, waxy sorghum, and "Amioca 85") produced loaves of inferior volume, having open crumb structure, and a very soft, moist, and sticky crumb immediately after baking. Of the modified starches, "Hercules 55," "Penford Gum 280," and "Vulca 30" gave the best bread. That the various starches had different hydration capacities was indicated by the absorption required to produce doughs of the desired consistency. These are given below:

Absorption	*Starch Substituted for Wheat Starch*
%	
58	None, rye
60	Corn, Hercules 55, Vulca 30
61	Cassava, Vulca 100
62	Oat, Penford gum 280
63	G 2
64	Nu Film, rice, waxy corn, Amioca 85
71	Waxy sorghum

The rates of crumb firming for breads containing natural starches with transition temperatures below that of wheat starch, namely cassava, rye, and oat starches, were of the same order of magnitude as that for bread containing only wheat starch (the rate for bread containing rye starch was somewhat greater but the difference was just significant at the 5% point). The average crumb firmness over the 69-hour storage period for breads containing rye and cassava starch was the same as that for bread containing only wheat starch, whereas the crumb firmness for bread containing oat starch was significantly lower.

Bread containing natural starches with transition temperatures greater than that of wheat starch, namely, waxy corn, corn, rice, and waxy sorghum starches, had a greater average crumb firmness and a greater crumb firming rate than bread containing only wheat starch. The crumb firming rates for the bread containing waxy corn and waxy sorghum starch were very high; in the case of the latter, compressi-

meter measurements could not be made after 31 hours' storage. These observations lend support to the view of Schoch and French (26) that it is the aggregation of the amylopectin fraction of starch rather than the retrogradation of the amylose which is primarily involved in the increase in the firmness of bread crumb on storage.

All the modified starches caused an increase in average crumb firmness. With the exception of "Vulca 100," they also caused an increase in the rate of crumb firming; however, the bread containing "Vulca 100" was already quite firm 4 hours after baking. Since the firming of bread crumb reaches a limiting value with time, any condition which produces a firm crumb initially would necessarily lead to a decreased firming rate.

General Discussion

These studies confirm the observations of baking technologists that strong flours produce bread of superior keeping qualities, as reflected by a soft crumb and a relatively slow change in this property upon storage. Microscopic examinations of bread crumb have shown that the partially swollen starch granules are embedded in a continuous protein membrane which surrounds the gas cells (5, 11, 12, 25). Increasing the protein content of the flour would tend to decrease the association between starch granules and thereby retard any changes in crumb firmness due to this factor. The ratio of protein to starch may also influence the crumb firming rate in another and more significant way. During baking the hydration capacity of the starch is increased by heat gelatinization, but there is insufficient moisture present to permit maximum hydration. Contrary to the belief of some of the early workers, however, the gluten retains a high swelling capacity (28) which Katz (19) has shown to remain unchanged during staling. The swelling power of the crumb as a whole, however, decreases with staling and the gluten may serve as a moisture reservoir to buffer the effects of changes in the hydration capacity of the starch.

The water-soluble proteins may contribute to this effect, since they decreased both the initial crumb firmness and the firming rate upon storage. Pence, Elder, and Mecham (23) have shown that the beneficial effect of the water-soluble fraction of flour with respect to loaf volume resides in the protein components of this fraction. In the present study doughs from which the water-solubles were omitted (preliminary unreported experiments) or used at a reduced level showed pronounced overdevelopment. Attempts by several workers (6, 13, 22) to demonstrate a high reducing titer in this fraction and to relate this with baking quality have been inconclusive. It is, therefore, not clear

whether the effects of the water-solubles are to be ascribed to reducing activity, proteolytic activity, or to other properties. They do, however, contribute to a properly conditioned dough in which the starch granules are enmeshed in an extensible film of protein.

The beneficial effect of the tailings fraction on crumb softness is doubtless associated with its high hydration capacity; the inclusion of this fraction increased the absorption and this would result in bread of higher moisture content and greater softness. It is perhaps surprising that this fraction had no influence on the rate of crumb-firming. However, it contains appreciable quantities of pentosans and proteins and its high hydration capacity is likely due primarily to these components rather than to the damaged starch granules which are present. The swelling power of the pentosans, in particular, would be little influenced by baking or storage of the bread and would tend to counteract any increase in crumb firmness due to changes in the water-sorption of the starch in this fraction.

Insufficient experimental data are available on the different natural and modified starches to permit a rational explanation of their effects on crumb firming. Among the natural unmodified starches, the temperature at which rapid swelling takes place (the transition temperature) is not necessarily related to the maximum hydration capacity. Nevertheless, maximum swelling cannot occur in such a concentrated starch-water system as bread because there is not sufficient water present.

The transition temperature is probably quite closely related to the gelatinization temperature, which is measured by noting the temperature range over which the granules in a suspension lose birefringence. This is indicated by the following gelatinization temperatures provided by T. J. Schoch[7] for several starches, some of which were used in this study:

	°C.		°C.
"Nu Film"	46–62	Corn	64–73
Potato	56–67	Corn (epichlorohydrin -treated)	65–74
"Penford 280"	59–69	Sorghum	68.5–75
Waxy maize	63–72		

The epichlorohydrin-treated derivative is typical of a cross-bonded starch and is analogous to the "Vulca" starches used in this study; potato starch is similar to Cassava in its resistance to retrogradation.

[7] Corn Products Refining Co., Argo, Ill. Private communication.

If the total increase in crumb-firmness from 4 to 69 hours' storage is considered, the starches can be divided into several groups:

1. The waxy starches. Starches of this type caused the greatest increase in firmness, probably because of their branched structure. These starches form long cohesive pastes in which the swollen granules adhere to one another.

2. Corn and rice starch. These starches have a lower content of amylopectin than the waxy starches, and upon gelatinization the granules remain fairly intact, so that most of the firming occurs within the granule.

3. The thin-boiling starches—"Nu Film," "Penford 280," and "Hercules 55." These starches probably disintegrate and dissolve to a considerable extent, and upon aggregation they would tend to give a continuous gel structure throughout the crumb. These starches give a substantially lower rate of crumb firming than "Amioca 85," which is also a soluble thin-boiling starch. Derivatization seems to have effected some reduction in the firming rate of corn starch.

4. Cassava. This starch is known to resist aggregation.

5. Cross-bonded starches. Bread containing these starches gave a low firming rate. They undergo very restricted swelling with little coherence between granules and the cross-bonding within the granules lessens their tendency to associate.

The fact that wheat starch yielded bread of lower crumb firmness and firming rate than bread in which one-third of the starch consisted of corn or rice would not be expected from the known properties of these starches.

These studies serve to supplement and confirm the results of somewhat similar experiments by Bechtel and Meisner (7), who found that bread made from mixtures of wheat gluten and potato, corn, and tapioca starch, respectively, firmed more rapidly than that made from a gluten-wheat starch mixture. They were unable to relate the differences in crumb firming rate to the granule sizes of the uncooked starch or to their gelatinization temperatures. The effects of the various starches on crumb firmness changes in bread may be related to differences in the degree of organization or crystallinity.

Acknowledgments

The authors are indebted to the American Maize Products Co., Roby, Indiana; Corn Products Refining Co., Argo, Illinois; National Starch Products Co., New York, N. Y.; Northern Utilization Research Branch, U. S. Department of Agriculture, Peoria, Ill.; Penick and Ford Co., Ltd., Cedar Rapids, Iowa, for supplying starch samples; and to T. J. Schoch, Corn Products Refining Co., for advice on their selection. Mr. H. Sorger-Domenigg assisted with the statistical calculations.

The authors also gratefully acknowledge the assistance of T. J. Schoch and Majel M. MacMasters (Northern Utilization Research Branch, Peoria, Ill.) in their endeavor to interpret the firmness data obtained on bread made with the various natural and modified starches.

Literature Cited

1. Alsberg, C. L. The stale bread problem. *In* Wheat studies. Food Res. Inst. **12(6):** 221–247 (1936).
2. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
3. Anker, C. A., and Geddes, W. F. Gelatinization studies upon wheat and other starches with the amylograph. Cereal Chem. **21:** 335–360 (1944).
4. Association of Official Agricultural Chemists. Official methods of analysis of the A.O.A.C. (7th ed.), p. 471. The Association: Washington, D.C. (1950).
5. Baker, J. C. Structure of the gas cell in bread dough. Cereal Chem. **18:** 34–41 (1941).
6. Baker, J. C., Parker, H. K., and Mize, M. D. The action of oxidizing agents on sulfhydryl compounds in dough. Cereal Chem. **21:** 97–107 (1944).
7. Bechtel, W. G., and Meisner, D. F. Present status of the theory of bread staling. Food Tech. **5:** 503–505 (1951).
8. Bechtel, W. G., Meisner, D. F., and Bradley, W. B. The effect of the crust on the staling of bread. Cereal Chem. **30:** 160–168 (1953).
9. Bice, C. W., and Geddes, W. F. Studies on bread staling. IV. Evaluation of methods for the measurement of changes which occur during bread staling. Cereal Chem. **26:** 440–465 (1949).
10. Bice, C. W., and Geddes, W. F. The role of starch in bread staling. *In* Starch and its derivatives (3rd ed.), ed. by J. A. Radley; Vol. II, pp. 202–242. Chapman and Hall: London (1953).
11. Burhans, M. E., and Clapp, J. A microscopic study of bread and dough. Cereal Chem. **19:** 196–216 (1942).
12. Butterworth, S. W., and Colbeck, W. J. Some photomicrographic studies of dough and bread structure. Cereal Chem. **15:** 475–488 (1938).
13. Chien, T. A study of some sulfhydryl groups in wheat flour. M. S. thesis, University of Minnesota (1949).
14. Clendenning, K. A., and Wright, D. E. Separation of starch and gluten. V. Problems in wheat starch manufacture arising from flour pentosans. Can. J. Research **F28:** 390–400 (1950).
15. Finney, K. F. Fractionating and reconstituting techniques as tools in wheat flour research. Cereal Chem. **20:** 381–397 (1943).
16. Geddes, W. F., and Bice, C. W. The role of starch in bread staling. Quartermaster Food and Container Inst. for the Armed Forces. Quartermaster Corps Report QMC 17–10. War Department, Office of the Quartermaster General, Washington, D.C. (Nov. 1, 1946).
17. Gilles, K. A. Studies on the gums derived from barley flour, wheat flour, and fresh and stale bread. Ph.D. thesis, University of Minnesota (1952).
18. Hutchinson, J. B. The staling and keeping quality of bread. Res. Assoc. of Brit. Flour Millers, Special Report No. 15, St. Albans, England (1936).
19. Katz, J. R. The change in swelling power of bread crumb during staling. Baker's Weekly (July 21, 1934).
20. MacMasters, M. M., and Hilbert, G. E. The composition of the "amylodextrin" fraction of wheat flour. Cereal Chem. **21:** 548–555 (1944).
21. Noznick, P. P., Merritt, P. P., and Geddes, W. F. Staling studies on breads containing waxy maize starch. Cereal Chem. **23:** 297–305 (1946).
22. Oelt, C. W. The influence of oxidizing and reducing agents on the baking characteristics of wheat flour. Ph.D. thesis, University of Minnesota (1944).
23. Pence, J. W., Elder, Angeline H., and Mecham, D. K. Some effects of soluble flour components on baking behavior. Cereal Chem. **28:** 94–104 (1951).
24. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. **16:** 780–792 (1939).
25. Sandstedt, R. M., Schaumburg, Lorene, and Fleming, J. The microscopic structure of bread and dough. Cereal Chem. **31:** 43–49 (1954).

26. SCHOCH, T. J., and FRENCH, D. Studies on bread staling. I. The role of starch. Cereal Chem. 24: 231–249 (1947).
27. STELLAR, W. R., and BAILEY, C. H. The relation of flour strength, soy flour, and temperature of storage to the staling of bread. Cereal Chem. 15: 391–401 (1938).
28. STOCKHAM, W. L. The capacity of wheat and mill products for moisture. N. D. Agr. Expt. Sta. Bull. 120 (1917).

GRAIN STORAGE STUDIES. XIV. CHANGES IN MOISTURE CONTENT, GERMINATION PERCENTAGE, AND MOLDINESS OF WHEAT SAMPLES STORED IN DIFFERENT PORTIONS OF BULK WHEAT IN COMMERCIAL BINS[1]

CLYDE M. CHRISTENSEN[2] AND ROBERT F. DRESCHER[3]

ABSTRACT

Small bags of wheat with a moisture content of 12% were buried at known locations in bins of commercial wheat of approximately 13% moisture as the bins were filled, and recovered when the bins were emptied. The moisture content of some of the samples recovered from two of the bins exceeded the average moisture content of the bulk by 3 to 4%, although the average moisture content of the samples was the same as that of the bulk. Circumstantial evidence indicated that the moisture content of the grain in some of the samples, at least in the outer portions of the seeds, exceeded 18–20% during part of the storage period. Storage molds increased in all of the samples stored in two of the bins and the viability of most of the samples decreased. Germ-damaged or "sick" wheat appeared in a few. The moisture content of samples stored throughout the bulk in the third bin remained essentially uniform; in a few of the samples in this bin molds increased slightly and viability decreased slightly.

There is abundant evidence that certain molds are a major factor in the deterioration of moist stored grain (3, 4, 5, 6, 9, 12). Practical grain storage men have at times claimed, however, that wheat and other grains in bulk storage have deteriorated at moisture contents too low to permit invasion of the seed by molds. This contention has been based on the "average" moisture contents for given bulks, and there has been little or no evidence as to the range in moisture contents that prevailed in such bulks. The moisture content of stored

[1] Manuscript received August 5, 1953. Paper No. 3035, Scientific Journal Series, Minnesota Agricultural Experiment Station. The work here reported was supported in part by a grant by Cargill, Inc., Minneapolis, Minn.
[2] Professor of Plant Pathology, Department of Plant Pathology and Agricultural Botany, University of Minnesota, St. Paul 1, Minnesota.
[3] Research Assistant, Department of Plant Pathology and Agricultural Botany, University of Minnesota, St. Paul 1, Minnesota.

grain may vary considerably from place to place in a given bulk (2, 7, 8, 12). Under certain conditions, particularly with sharp differences in temperature between different portions of a given bulk, moisture may move slowly or rapidly from one portion of the grain to another (2, 7, 8, 12). In practice, allowances usually are made for a certain amount of variation from the average moisture content of the bulk. Whether these allowances, based on long experience in practical grain storage, actually are sufficient to include the range in moisture content that may occur in a given bin, is not known.

That they may not be was suggested by the following: In March, 1952, eighteen samples were taken by bucket probe, at depths of 5, 15, and 30 ft., in six different places in a bulk of wheat stored in a quonset-type bin, with an average moisture content of 13.2%. Seven of the samples so taken had a moisture content in excess of 13.5%, and four of them had a moisture content in excess of 14.5%. All of the samples were placed in screw-topped bottles, which they filled to the top; the bottles were sealed with rubber liners inside the screw caps and stored in the laboratory. Within a year, microscopically visible molds had developed on seven of the 18 samples, some of these with a moisture content below 14%. In some of the seeds with no microscopically visible external molds, mycelium had developed extensively under the pericarps. These preliminary investigations suggested that tests designed to explore more thoroughly the range in moisture content and mold invasion of bulk stored wheat might be of value, and led to the study reported here.

Materials and Methods

Wheat Samples Buried in Bulk Grain. Most of the samples consisted of hard red spring wheat, variety Marquis, grown in Montana in 1951. This was chosen, from about 30 samples tested, primarily because of its relative freedom from storage molds and general excellent condition and high grade. It had a moisture content of 11.0 to 12.0%, a germination of 96%, and a mold count of less than 1000 per g.; and when 600 seeds were surface-disinfected and cultured on malt-salt agar, from 2 to 4% yielded *Aspergillus glaucus* but no other storage molds were present in the seed. In one bin, samples of a No. 2 grade northern spring wheat were buried along with the Montana Marquis; this wheat had a germination of 94% and a mold count of 4500 per g., made up equally of *Penicillium*, *A. glaucus*, and *A. candidus*. When surface-disinfected and cultured on malt-salt agar, 18% of the seeds yielded *A. glaucus*, and 6% *A. candidus*. The aim in using this wheat was to compare the storage behavior of what was

presumed to be a fairly typical grade 2 hard red spring wheat with that of the high-quality hard red spring wheat.

Location and Description of the Bins. Bins 1 and 2 were located at Richmond, Virginia. They were concrete cylinders 15 ft. in diameter and 140 ft. high. When filled to within 4 ft. of the top each contained about 15,000 bu. Bin 3, located in Minneapolis, was 40 ft. in diameter and 90 ft. high and contained 45,000 bu. filled to a depth of 45 ft.

Description of the Bulk Wheat in the Bins. Bin 1, in Richmond, was filled with No. 2 soft red winter wheat on August 7, 1952. All of the wheat came from the vicinity of Richmond, and had been harvested shortly before it was binned. Nearly 100% of the seeds were invaded with various molds at the time the wheat came to the terminal, but the germination of the wheat was 85%, indicating that it was in fairly good condition. The moisture content of the grain was approximately 13.0%, and its temperature was from 90 to 95°F., which was the temperature of the outside air at the time. The bin was emptied on November 27, 1952, after a storage period of approximately 4 months.

Bin 2 contained wheat nearly identical with that of bin 1. It was filled in January, 1953, and emptied approximately 1 month later.

Bin 3 was loaded with 45,000 bu. of No. 1 dark northern spring wheat of the 1952 harvest that had been stored since shortly after harvest in three smaller bins at the same elevator. It had a moisture content of 13.0% and a temperature of about 55°F. It germinated 90%, and had a mold count of 16,000 per g., of which 90% was *Aspergillus glaucus;* when surface-disinfected and cultured on malt-salt agar, 26% of the seeds yielded *A. glaucus.* The bin was emptied 3 weeks after it had been filled.

Placement of Sample Bags in the Bulk Grain. The wheat to be buried was placed in small muslin grain sample bags, approximately 1 lb. per bag, and the bags numbered with India ink for identification. At Richmond, four steel cables were fastened to the roof and floor of each bin; three of the cables equidistant from one another, 4 ft. in from the wall of the bin, and the fourth at the center. The sample bags were fastened to these with tape as the bins were being filled. The bags were placed at vertical intervals of 13.5 ft. along each cable, and their position was known accurately. In the test in Minneapolis, the bags were thrown into the bin through manholes at the top of the bin, as the bin was being filled. At each of eight levels, a sample was dropped near the outside wall, one near the inside wall, and one close to the center. In the Minneapolis bin, each sample comprised two bags, one

of them containing Montana Marquis, the other No. 2 hard red spring wheat from a commercial parcel in the same elevator. The two bags at each location were tied tightly together throughout their length.

Moisture. All moisture determinations were by the two-stage, air-oven method specified in Cereal Laboratory Methods (1), and are given on a wet-weight basis.

Germination. One hundred to two hundred seeds were placed on moist germination paper, incubated at room temperature for 5 days, and the number of seedlings counted.

Moldiness. Three methods were used to measure the degree of moldiness or mold invasion of the seeds. The mold count was made as described by Bottomley, Christensen, and Geddes (3); this measures primarily the number of viable spores, but does not measure amount of growing mycelium within the interior of the seed. To determine the percent of seeds deeply infected with storage molds, the seed was surface-disinfected in 1.0% sodium hypochlorite plus detergent for 1 minute and rinsed in sterile water three times; then 50 to 100 seeds were placed on malt-salt agar in Petri dishes. After incubation for 5–7 days, the number and kinds of molds growing from the seed were recorded. This technic measures the number of seeds deeply invaded by storage molds, but does not evaluate the amount or extent of such mycelium. For the "delayed" or incubation mold count, 25 g. of seed were placed in a 90-mm. Petri dish, an amount of 1% sodium hypochlorite solution, containing a small amount of detergent, sufficient to cover the seed was added, the dish was then covered and shaken vigorously for 15–20 seconds, and the sodium hypochlorite solution was poured off; sterile water was then added to cover the seed and poured off; the seeds were covered with a sterile solution of 2% malt extract and 10% sodium chloride in distilled water. The seeds were steeped in this for 1 hour and the solution poured off; the seeds were then incubated for 3 days, after which a mold count was made in the regular way. This treatment removes or kills external spores and kills the mycelium immediately beneath the pericarp, but allows the mycelium deeper in the seeds to produce spores. The 10% salt solution reduces or inhibits the sporulation of any but storage molds. This technic was devised during the present study in an attempt to measure the amount of mycelium of storage molds growing within the seeds. Under some conditions sporulation may be absent or scanty even though mycelium is abundant within the seeds; this technic aimed to detect and measure such hidden internal growth. This method supplements but does not replace the first two methods listed.

Germ-Damage. The percentage of germ-damaged cereals was de-

termined by inspectors of the Minnesota Grain Inspection Department, Minneapolis, Minn.

Results

The major results are summarized in Tables I to IV.

TABLE I

MOISTURE CONTENT, GERMINATION, AND MOLDINESS OF SAMPLES OF MONTANA MARQUIS WHEAT BURIED IN BIN 1 AT RICHMOND, VA., AUGUST THROUGH NOVEMBER, 1952

Location Cable No.	Depth	Moisture Content	Germination	Surface-Disinfected Seeds Yielding		Mold Count per g. (in thousands)		
				Aspergillus glaucus	Aspergillus flavus	Total	Aspergillus glaucus	Aspergillus flavus
	Ft.	%	%	%	%			
1	135.0	12.6	81	8	0	1.5	..	..
	121.5	12.3	71	66	0	2.0	1.5	..
	108.0	12.3	31	88	0	5.0	2.0	0.5
	94.5	12.4	53	88	0	1.0	1.0	..
	81.0	12.9	63	84	2	7.0	4.0	1.0
	67.5	12.2	55	68	12	11.0	9.0	1.0
	54.0	12.5	41	86	2	20.0	3.0	1.0
	40.5	13.4	70	80	0	4.5	0.5	..
	27.0	14.0	48	90	0	30.0	20.0	..
	13.5	14.5	0	0	88	470.0	320.0	..
2	135.0	10.6	55	24	0	3.0	2.0	0.5
	121.5	10.7	87	20	0	3.5	2.5	..
	108.0	10.9	68	88	0	15.0	10.0	..
	94.5	10.3	65	48	0	10.0	7.5	2.5
	81.0	11.0	84	84	0	55.0	40.0	10.0
	67.5	12.1	84	64	6	45.0	40.0	..
	54.0	12.8	85	62	0	45.0	45.0	..
	40.5	11.4	70	52	2	35.0	35.0	..
	27.0	11.2	71	88	0	1.0	0.5	..
	13.5	10.8	88	64	2	0.5	0.5	..
3	135.0	11.6	91	68	2	3.0	1.5	..
	121.5	11.0	88	54	0	4.0	4.0	..
	108.0	11.0	87	70	4	3.5	3.5	..
	94.5	11.6	76	80	0	5.0	..	..
	81.0[1]							
	67.5	11.8	93	70	4	7.0	2.5	1.0
	54.0	11.2	86	46	22	25.0	25.0	..
	40.5	11.2	82	74	0	3.5	1.5	..
	27.0	12.1	97	20	0	0.5	0.5	..
	13.5	11.6	91	22	0	0.5	0.5	..
4	135.0	10.8	94	38	2	3.0	0.5	..
	121.5	13.2	44	2	96	710.0	360.0	250.0
	108.0	14.4	46	8	100	135.0	68.0	13.0
	94.5	12.1	86	80	2	12.5	10.0	1.5
	81.0	12.3	91	68	0	14.0	5.0	2.0
	67.5	12.6	88	58	8	12.0	5.5	2.5
	54.0	11.9	93	66	0	19.0	5.0	3.0
	40.5	14.1	53	58	96	78.0	78.0	..
	27.0	12.0	85	58	0	4.0	4.0	..
	13.5	15.7	6	44	98	282.0	100.0	140.0
Average		12.1	70%					

[1] Missing.

Discussion

Moisture Content. The moisture content of the 39 samples recovered from bin 1 (Table I) averaged 12.1%, which agreed closely with the average moisture content of the entire bulk as determined when the bin was emptied. The moisture content of the individual samples ranged from 10.3% to 15.7%. In bin 2 (Table II), the moisture con-

TABLE II

MOISTURE CONTENT, GERMINATION, MOLDINESS, AND DAMAGED SEED IN SAMPLES OF MONTANA MARQUIS WHEAT BURIED IN BIN 2 AT RICHMOND, VA., JANUARY 10 TO FEBRUARY 15, 1953

Cable No.	Sample Location Depth	Moisture Content	Germination	Damaged or Sick Grain	Surface Disinfected Seeds Yielding		Mold Count per g. (in thousands)		
					Aspergillus glaucus	Aspergillus flavus	Total	Aspergillus glaucus	Aspergillus flavus
	Ft.	%	%	%	%	%			
1	135.0	12.1	80	0	78	26	22.0	20.0	..
	121.5	14.3	64	0	44	10	15.0	10.0	0.5
	108.0	12.1	62	3	82	10	16.0	12.0	..
	94.5	12.3	56	70	66	34	23.0	18.0	1.0
	81.0	12.3	67	0	82	46	19.0	16.0	..
	67.5	12.3	72	1	56	6	15.0	10.0	0.5
	54.0	12.2	65	0	84	22	9.0	4.5	0.5
	40.5	16.5	0	100	58	22	1200.0	1000.0	..
	27.0	12.2	40	1	58	4	19.0	9.0	8.0
	13.5	14.4	0	..	16	100	700.0	540.0	160.0
2	135.0	12.1	56	0	48	24	19.0	17.0	2.0
	121.5	12.4	49	0	62	34	48.0	37.0	3.0
	108.0	11.4	64	0	58	8	8.0	6.0	..
	94.5	12.2	52	0	66	20	24.0	15.0	8.5
	81.0	13.5	0	38	48	70	470.0	457.0	13.0
	67.5	11.8	49	0	68	56	4.0	4.0	..
	54.0	12.4	26	..	86	22	4.0	3.5	0.5
	40.5	12.4	21	COFO	80	2	1.5	1.0	0.5
	27.0	12.5	16	..	4	100	94.0	4.0	90.0
	13.5	16.8	51	22	92	2	120.0	120.0	..
3	135.0	12.2	71	..	60	3	5.5	5.0	0.5
	121.5	12.2	68	0	48	8	3.5	3.0	..
	108.0	12.5	34	0	70	22	4.5	0.5	1.5
	94.5	12.2	61	0	50	44	9.5	5.0	..
	81.0[1]								
	67.5	12.9	83	1	84	14	2.0	1.0	..
	54.0	12.2	64	0	28	1	268.0	3.0	29.0
	40.5	12.1	79	0	72	4	327.0	100.0	27.0
	27.0	12.7	82	0	64	0	130.0	65.0	5.0
	13.5	15.6	41	0	54	36	245.0	75.0	25.0
4	135.0	12.3	66	0	40	8	310.0	150.0	30.0
	121.5	11.8	80	0	28	4	153.0	75.0	20.0
	108.0	14.0	55	..	12	100	330.0	120.0	110.0
	94.5	12.4	63	0	22	0	92.0	50.0	6.0
	81.0	12.5	53	..	12	100	368.0	100.0	134.0
	67.5	12.2	80	0	100	20	226.0	75.0	12.0
	54.0	12.6	83	2	10	28	293.0	140.0	15.0
	40.5	12.3	77	0	14	0	130.0	13.0	15.0
	27.0	12.3	77	..	44	0	288.0	96.0	17.0
	13.5[1]								
Average		13.1	55%						

[1] Lost.

tent of the 38 samples recovered averaged 13.1% and ranged from 11.8% to 16.8%. Obviously, the moisture content as determined on the average sample at the elevator, on which an elevator operator would base his opinion as to the storage risk of the entire lot of grain in the bin, did not give adequate information on the range of moisture contents within these particular bulks. No clear and definite trend of high or low moisture content of samples with position in the bin could be detected, either horizontally or vertically. The average moisture content of the 32 samples recovered from the Minneapolis bin was 12.8%, and the range was from 11.8% to 13.5% (Table IV). This small range in moisture content undoubtedly reflected the stable

TABLE III

VIABILITY AND MOLDINESS OF SIX SAMPLES OF LOWEST VIABILITY AND SIX SAMPLES OF HIGHEST VIABILITY FROM BIN 1

	Viability	Surface-Disinfected Seeds Yielding		Mold Count per g. (in thousands)	
		Aspergillus glaucus	Aspergillus flavus	Aspergillus glaucus	Aspergillus flavus
		%	%		
Six samples of lowest viability					
	0	0	88	320	0.0
	6	44	98	100	140.0
	31	88	0	5	0.5
	41	86	2	3	1.0
	44	2	96	360	250.0
	46	8	100	68	13.0
Average	25	38	64	142	67.0
Six samples of highest viability					
	91	68	2.0	1.5	0
	91	22	0.0	0.5	0
	91	68	0.0	5.0	2
	93	66	0.0	5.0	3
	94	38	2.0	0.5	0
	97	20	0.0	0.5	0
Average	92	47	0.66	2.0	1

condition of the grain; it was of uniformly high quality and low temperature when stored, and remained so through the short period of storage.

The data on moldiness of the samples buried in the bins indicated fairly conclusively that fluctuations in moisture content, even greater than those determined in the samples as the bins were emptied, must have prevailed for some time in certain portions of the bin. For example, in bin 1, seven samples had from 12 to 100% of the seeds invaded by *Aspergillus flavus*. It is a well-established fact that this fungus will not invade wheat until the moisture content of the seed is between 17 and 18% (5, 11, 12). Eleven, or nearly 30%, of the samples yielded *Mucor* from a high percentage of the surface-disinfected seeds. This fungus, as shown by Snow (11, 12), requires a relative humidity of close to 95% before it will grow. At some time during the storage period, at least the outer portion of the seeds at these locations must have had a moisture content in excess of 20%. When the bin was emptied, the sample at the 67.5-ft. depth along cable 1 had a moisture content of 12.2%; the sample at the 54-ft. level along cable 3 had a moisture content of 11.2%, and the sample at the 121.5-ft. level along cable 4 had a moisture content of 13.2%. All of these moisture contents are well below those necessary to permit the seed to be invaded

TABLE IV

MOISTURE CONTENT, VIABILITY, AND MOLDINESS OF SAMPLES OF TWO DIFFERENT PARCELS OF WHEAT BURIED AT DIFFERENT PLACES FOR THREE WEEKS IN A BIN OF NO. 1 NORTHERN SPRING WHEAT IN MINNEAPOLIS, MARCH 25 TO APRIL 10, 1953

Depth	Moisture Content	Viability	Surface-Disinfected Seeds Yielding		Mold Count in Thousands/g.			
					Seeds Dry		Seeds Incubated 3 Days	
			Asper. glaucus	Asper. flavus	Asper. glaucus	Asper. flavus	Asper. glaucus	Asper. flavus
Outside [4]								
40 A [1]	13.3	82	8	2	1.5	0	800	0
B	12.0	83	10	0				640
36 A	12.9	82	26	0	1.5	0	0	0
B	12.6	84	10	4	0.0	0		0
30								
26 [2]								
20								
16 A	13.1	75	12	0	1.5	0	500	0
B	12.9	89	8	0	0.5	0	0	0
10 A	13.2	79	8	0	1.5	0	250	0
B	12.5	85	14	0	0.5	0	0	0
6 A	13.1	67	18	6	0.0	0	68	0
B	12.4	82	0	4	1.5	0	0	0
Center [4]								
40 A	13.2	75	8	0	2.5	0	2,800	0
B	11.8	87	10	0	1.5	0	1,560	0
36 A	13.5	85	8	0	1.5	0	1,340	0
B	12.3	91	12	0	0.5	0	350	0
30 A	13.2	81	16	0	0	0	— [3]	—
B	12.3	85	8	0	0	0	—	—
26 A	13.3	87	8	0	1.0	0	—	—
B	12.6	77	4	0	0.0	0	—	—
20 [2]								
16 [2]								
10 A	12.9	85	10	2	2.5	0	670	380
B	12.8	85	6	0	0.0	0	480	20
6 A	12.8	85	14	0	1.0	0	140	—
B	11.9	74	0	0	0.0	0	960	—
Inside [4]								
40 A	13.0	72	42	0	1.0	0	800	—
B	12.3	82	8	0	0.0	0	3	—
36 A	13.5	76	54	0	0.0	0		
B	12.2	93	14	0	0.0	0		
30 A	12.8	81	38	20	1.0	0	184	—
B	12.3	86	10	0	0.0	0	350	—
26 [2]								
20 A	13.4	83	20	0	0.5	0	2	—
B	12.2	75	14	0	0.0	0	1	—
16 A	13.3	83	14	0	1.0	0	1,080	—
B	12.8	87	22	0	0.0	0	4	—
10 A	12.7	71	24	0	0.0	0	660	—
B	12.7	89	12	0	0.0	0	150	—
6 A	13.0	68	46	4	1.0	0	350	—
B	11.8	70	22	0	0.0	0	29	—
Average								
A	13.1	80	21				689	
B	12.3	84	10				283	

[1] A = No. 2 northern spring; B = Montana Marquis.
[2] Samples lost.
[3] Not analyzed.
[4] Location, vertical.

by *A. flavus*. Yet all of these samples had been invaded by this fungus. At some time during the storage period the moisture content of these samples of Marquis wheat almost certainly exceeded 17%, at least in the outer portions of the seed. The fact that seeds of 100% of

the samples were invaded by either *A. glaucus* or *A. flavus* during the storage period is evidence that all samples, at some time during storage, had a moisture content above 14% for at least several weeks. The same is true, to an even greater extent, of the samples stored in bin 2 at Richmond, even though the storage period was only approximately one month.

Germination. In bin 1 at Richmond, the germination of the grain in the sample bags decreased from an original 96% to an average of 70%. Seven of the samples had an average germination of less than 50%. In bin 2 at Richmond, the germination after storage for approximately 1 month averaged 55%, and 11 of the 38 samples had a germination of less than 50%. The samples stored in the bin in Minneapolis decreased only 10–15% in germination, although even in this bulk of high-quality grain of low temperature and apparently uniform moisture content there was some increase in storage molds and some decrease in germination of the test samples during the short period of storage.

Germ-Damaged Wheat. The percentages of germ-damaged or so-called "sick" wheat were determined only in a portion of the test samples in one bin at Richmond and are recorded in Table II. In most of the samples in which sick wheat appeared, the germination had decreased significantly, and either the seeds had a high mold count, or a high percentage of the seeds, after surface-disinfection, yielded storage molds. There was no obvious correlation between decrease in germination, or mold invasion, and the percentage of sick wheat. It seems, however, that mold invasion of the seed and decrease in germination precede the appearance of sick wheat detectable by the visual and, admittedly, rather crude method now used to detect this sort of deterioration.

Mold Invasion. All of the samples in both of the bins at Richmond were lightly to heavily invaded by molds during the storage period. As stated above, the degree of invasion by *A. glaucus* and *A. flavus* was greater than could be accounted for by the moisture contents of the samples when the bins were emptied. The only reasonable explanation is that higher moisture contents prevailed at nearly all positions in these bins where samples were stored, at various times during storage, than was indicated by the moisture contents of the samples as the bins were emptied. The averages given in Table III indicate that invasion of the seed by *A. flavus* is likely to be more injurious than invasion by *A. glaucus*. The work of Thomas (13) indicates that *A. flavus* might be rather toxic to seeds. However, both of these fungi appear to invade the seed and kill the living germ.

It was apparent that at least a significant proportion of the deterioration encountered in these bins of commercially stored wheat followed this course: 1) Increase in moisture content, in certain portions of the bulk, significantly above the average moisture content of the bin; 2) invasion of the seed by storage molds accompanied by 3) decrease in germination sometimes followed by 4) the appearance of sick wheat.

Wheat Quality. In the Minneapolis bin, two bags of wheat were stored at each position; one of them contained Montana Marquis, the other a parcel of No. 2 spring wheat that was moderately invaded by storage molds prior to storage, even though its germination was 94%. The two bags were tied together tightly throughout their length. As the bin was emptied, the moisture content of the samples of No. 2 wheat averaged nearly 1% above that of the samples of Montana Marquis; the germination of the No. 2 wheat at the end of the test had decreased to an average of 89%, as compared with 84% for Montana Marquis; and the samples of No. 2 wheat had been somewhat more invaded by *A. glaucus* than the samples of Marquis, as indicated by both the surface disinfection and culturing technic and the delayed mold count. Thus, by all the criteria used to evaluate deterioration, the samples of No. 2 wheat had deteriorated somewhat more during the short period of storage than had the samples of No. 1 wheat. Whether this was a function of quality *per se*, of previous mold invasion of the samples, or of other factors, is not known. It suggests, at least, that wheat of lower grades might be inclined to deteriorate more rapidly, under identical conditions of storage, than those of higher grades. This might well be considered in the general practice of mixing or blending parcels of different grade and quality to make a supposedly uniform bulk.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minn. (1947).
2. Anderson, J. A., Babbitt, J. D., and Meredith, W. O. S. The effect of temperature differential on the moisture content of stored wheat. Can. J. Research **21C:** 297–306 (1943).
3. Bottomley, R. A., Christensen, C. M., and Geddes, W. F. Grain storage studies. X. The influence of aeration, time, and moisture content on fat acidity, nonreducing sugars, and mold flora of stored yellow corn. Cereal Chem. **29:** 53–64 (1952).
4. Carter, E. P., and Young, G. Y. Role of fungi in the heating of moist wheat. U.S. Dept. Agr. Circ. 838 (1950).
5. Christensen, C. M., and Gordon, Dorothy R. The mold flora of stored wheat and corn and its relation to heating of moist grain. Cereal Chem. **25:** 40–51 (1948).

6. GILMAN, J. C., and SEMENIUK, G. Mold microflora in stored grain and its role in the deterioration process. Trans. Amer. Assoc. Cereal Chem. **6:** 108–112 (1948).
7. OXLEY, T. A. The movement of heat and water in stored grain. Trans. Amer. Assoc. Cereal Chem. **6:** 84–100 (1948).
8. OXLEY, T. A. The scientific principles of grain storage. (103 pp.) Northern Pub. Co., Ltd.: Liverpool, England (1948).
9. RAMSTAD, P. E., and GEDDES, W. F. The respiration and storage behavior of soybeans. Minn. Agr. Exp. Sta. Tech. Bull. 156 (1942).
10. SEMENIUK, G., and GILMAN, J. C. Relation of molds to the deterioration of corn in storage, a review. Proc. Iowa Acad. Sci. (for 1944) **51:** 265–280 (Dec. 1944).
11. SNOW, D. Mould deterioration of feeding stuffs in relation to humidity of storage. Part III. The isolation of mould species from feeding stuffs stored at different humidities. Ann. Appl. Biol. **32:** 40–44 (1945).
12. SNOW, D. The germination of mould spores at controlled humidities. Ann. Appl. Biol. **36:** 1–17 (1949).
13. THOMAS, R. C. The role of certain fungi in the "sick wheat" problem. Ohio Agr. Exp. Sta. Bi-monthly Bull. **22:** 43–45 (1937).

RELATION BETWEEN PROTEIN CONTENT OF CORN AND CONCENTRATION OF AMINO ACIDS AND NICOTINIC ACID[1]

LAURA M. FLYNN, MARCUS S. ZUBER, DELBERT H. LEWEKE, ROBERT B. GRAINGER, AND ALBERT G. HOGAN[2]

ABSTRACT

Microbiological assays were made of 13 samples of low-protein corn and 15 samples of high-protein corn for certain nutritionally essential amino acids and nicotinic acid. The range of protein in the low-protein corn was 8.8 to 10.3% (av., 9.9%), and protein in the samples of high-protein corn ranged from 12.8 to 15.4% (av., 14.3%). Microbiological assays gave these average results for low-protein corn and high-protein corn, respectively: 1) tryptophan, 87 and 99 mg.%; 2) lysine, 314 and 380 mg.%; 3) methionine, 199 and 239 mg. %; and 4) nicotinic acid, 2.53 and 2.40 mg. %. The average results of chemical determinations of cystine in the low-protein corn and the high-protein corn were, respectively, 144 and 182 mg. %. When the protein content of the corn increased, the amount of each nutrient per g. protein decreased. Average amounts of the nutrient, per g. of protein in low-protein corn and in high-protein corn, were, respectively: 1) tryptophan, 8.9 and 6.9 mg.; 2) lysine, 31.1 and 26.6 mg.; 3) methionine, 20.3 and 18.1 mg.; 4) cystine, 14.6 and 12.8 mg.; and 5) nicotinic acid, 0.258 and 0.169 mg.

The extensive investigations that have been carried on in an effort to improve the nutritional value of corn through plant breeding have been summarized in recent issues of *Nutrition Reviews* (1, 2, 3, 4). This paper reports the amounts of the essential amino acids tryptophan, lysine, methionine, and cystine, in corn samples of different protein content. The samples were assayed for nicotinic acid also, because of the tryptophan-nicotinic acid relationship.

Materials and Methods

Samples. The samples assayed for amino acids and nicotinic acid consisted of the grain from self-pollinated ears of an open-pollinated variety, Jumbo Yellow Dent Corn, obtained from the Corn Breeding Project of the Field Crops Department of the University of Missouri. The soil upon which these samples were produced had received an application of 200 lb. per acre of 8:8:8 fertilizer, furnishing accordingly 16 lb. of added nitrogen per acre. Since the ears represented an unselected group from a heterogeneous population a range in protein content might be expected. The corn kernels were ground in a Wiley

[1] Manuscript received June 4, 1951. Contribution from the Departments of Agricultural Chemistry and Field Crops, Section of Cereal Crops and Diseases, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture cooperating. Missouri University Agricultural Experiment Station Journal Series No. 1240. Presented at the meeting of the American Chemical Society, at Chicago, Ill., September 5, 1950.

[2] Departments of Agricultural Chemistry and Field Crops, University of Missouri, Columbia.

mill to pass through a sieve with 2-mm. openings. Corn and casein obtained from commercial sources were used as check samples.

Kjeldahl nitrogen determinations were made on 153 corn samples, and the factor 6.25 was used to convert nitrogen to crude protein. The 15 highest in crude protein and the 13 lowest in crude protein were assayed for the four amino acids and for nicotinic acid.

Methods of Hydrolysis. Hydrolysates for assay (except for cystine) were prepared by autoclaving the samples at 15 lb. pressure, in acid or in alkali according to the routines described here. Enzymes were unsatisfactory in the extraction of tryptophan from low-protein corn, probably because the correction for tryptophan in the enzyme was often over 50% of the total amount. Alkaline hydrolysis, a modification of the method of Greene and Black (16) was accordingly used in preparing extracts of tryptophan from both high- and low-protein corn, and 1-g. samples were autoclaved in 10 ml. of 4.2*N* barium hydroxide solution for 7 hours. One-gram samples for assay of lysine and methionine were autoclaved in 20 ml. of 2 *N* hydrochloric acid solution for 10 hours, a modification of the hydrolysis procedure of Barton-Wright (6). For the extraction of nicotinic acid, 1-g. samples were autoclaved in 100 ml. of *N* sulfuric acid solution for 30 minutes as recommended by Strong (31).

For the assay of cystine, 5-g. samples were dried overnight in a vacuum oven, subjected to extraction with diethyl ether for 24 hours, and freed from most of their starch by a saliva digestion during 72 hours as recommended by Doty (10). The residues from the enzyme digestion were washed with warm water, dried overnight, and hydrolyzed by refluxing for 5 hours, with 15 ml. of 18% hydrochloric acid solution, as suggested by Block and Bolling (8). Each hydrolysate was transferred to a small evaporating dish, heated over a steam bath until the residue was syrupy in consistency, dissolved in water, brought to a volume of 50 ml., and filtered. Doty (10) found that approximately 20% of the original weight of the ground fat-free corn remained in the residue after saliva digestion, and 45% of the residue was protein. Similar determinations were not made on these samples.

Assay Methods. Information concerning details of methodology in the microbiological assays is presented in Table I. The basal media, which represented modifications of media described in the literature, permitted excellent growth and dose-response curves were linear over a wide range. The quantities of standard solutions and of unknowns were so chosen that they were in geometric progression. Data showing ml. of acid produced were plotted on log-log paper, and the results were calculated by the method suggested by Wood (33).

TABLE I

DETAILS OF MICROBIOLOGICAL ASSAY METHODS

Assay	Medium Modified	Test Organism[a]	Range of Standard
			μg.
Tryptophan	Flynn *et al.* (12)	*Lactobacillus arabinosus*	0.625 to 20.0
Lysine	Riesen *et al.* (27) (Omit oxidized peptone, substitute crystalline amino acids. Add 15 mg. xanthine per 100 ml. basal medium.)	*Leuconostoc mesenteroides*–P-60	4 to 128
Methionine	Riesen *et al.* (27) (Omit oxidized peptone, substitute crystalline amino acids.)	*Leuconostoc mesenteroides*–P-60	1 to 32
Nicotinic acid	Flynn *et al.* (12).	*Lactobacillus arabinosus*	0.01 to 1.6

[a] All cultures were titrated after 72 hours' incubation at 33°C.

Attempts were made to determine the cystine in whole dry corn by a microbiological procedure, using *Lactobacillus arabinosus* as the test organism. The assay method was unsatisfactory and the samples were assayed by a chemical method. Hydrolysates prepared for assay of cystine were decolorized with Darco, and assayed according to the Block and Bolling modification of the Winterstein-Folin Reaction (8). The phospho-18-tungstic acid reagent was prepared according to the method of Folin (14), from sodium tungstate free of molybdenum. After development of the color, the sample extracts were read against the "blanks" in a photoelectric colorimeter, using a filter transmitting light of wavelength 520 mμ.

Results and Discussion

Nutrients in Whole Dry Corn Grain and in Protein. Data from individual assays appear in Table II. The average concentrations of the nutrients assayed in 13 samples of low-protein corn (range, 8.8 to 10.3%) and in 15 samples of high-protein corn (range, 12.8 to 15.4%) were: 1) crude protein, 9.9 and 14.3%; 2) tryptophan, 87 and 99 mg.%; 3) lysine, 314 and 380 mg.%; 4) methionine, 199 and 239 mg.%; 5) cystine, 144 and 182 mg.%; and 6) nicotinic acid, 2.53 and 2.40 mg.%.

The assays for methionine and cystine are considered less dependable than the assays for other nutrients. The methods of determining free cystine and methionine are reasonably satisfactory, but the validity

TABLE II

AMINO ACIDS AND NICOTINIC ACID IN CORN GRAIN PER 100 G. DRY MATTER

Crude Protein	Tryptophan	Lysine	Methionine	Cystine	Nicotinic Acid
%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
8.8	80	302	188	134	2.50
9.4	79	307	210	149	2.38
9.5	90	327	261	146	2.96
9.6	83	307	210	151	1.95
9.7	79	301	172	172	3.36
9.8	83	302	173	127	2.36
9.9	83	286	176	143	2.43
9.9	88	361	197	126	2.74
10.2	..	347	156	107	2.20
10.3	91	285	228	174	1.95
10.3	111	284	157	160	2.39
10.3	87	272	257	142	2.82
10.3	..	294	205	139	2.87
12.6[a]	86[a]	294[a]	288[a]	227[a]	..
12.8	100	..	203	182	3.38
13.8	97	410	259	198	2.37
13.9	96	360	255	214	2.50
13.9	99	390	207	173	2.02
14.0	89	360	213	..	..
14.1	103	360	312	190	1.97
14.1	96	410	232	174	2.09
14.1	103	330	213	150	2.96
14.3	100	320	310	178	2.36
14.3	94	410	304	209	2.38
14.5	104	400	302	173	2.19
14.6	101	390	242	183	2.51
14.6	104	400	212	152	1.85
14.9	103	380	311	182	2.51
15.3	94	410	302	195	2.50

[a] Not included in statistical calculations.

of the extraction procedures has not been established. Figures for cystine and methionine in Tables II, III, and IV should accordingly be considered minimal values. One would expect losses of the sulfur amino acids in hydrolysis to be more extensive in the samples from the low-protein group because low-protein corn contains proportionally more carbohydrates.

Examination of the data reported here shows that the high-protein corn contained more tryptophan, lysine, methionine, and cystine than the low-protein corn. If one considers the concentration of each nutrient per g. protein, the changes accompanying an increase in protein are more evident. The amounts of amino acids and nicotinic acid per g. of crude protein are shown in Table III. Average amounts of the nutrients per g. of protein in low-protein corn and in high-protein

TABLE III

AMINO ACIDS AND NICOTINIC ACID PER UNIT CRUDE PROTEIN IN 29 SAMPLES OF CORN GRAIN
(Comparison with Data in Literature)

Protein Content of Moisture-Free Corn	Amino Acids in Crude Corn Protein				Nicotinic Acid per 100 g. Crude Protein	Source of Data (Authors)
	Trypto-phan	Lysine	Methio-nine	Cystine		
%	%	%	%	%	*mg.*	
8.8	0.91	3.42	2.14	1.53	28.4	
9.4	0.84	3.28	2.25	1.59	25.3	
9.5	0.95	3.44	2.75	1.53	31.1	
9.6	0.87	3.20	2.20	1.58	20.4	
9.7	0.81	3.09	1.77	1.76	34.5	
9.8	0.85	3.09	1.77	1.30	24.1	
9.9	0.84	2.89	1.78	1.44	24.6	
9.9	0.89	3.65	1.99	1.28	27.6	
10.2	...	3.39	1.53	1.05	21.5	
10.3	0.89	2.78	2.22	1.69	19.0	
10.3	1.08	2.76	1.53	1.56	23.2	
10.3	0.84	2.64	2.49	1.38	27.3	
10.3	...	2.85	1.99	1.34	27.7	
12.6[a]	0.68	...	...	1.81[a]	...	
12.8	0.78	...	...	1.42	26.4	
13.8	0.70	2.97	1.88	1.44	17.1	
13.9	0.68	2.59	1.83	1.54	18.0	
13.9	0.71	2.80	1.48	1.24	14.5	
14.0	0.63	2.57	1.52	...	...	
14.1	0.73	2.56	2.22	1.35	14.0	
14.1	0.68	2.91	1.65	1.24	14.8	
14.1	0.74	2.34	1.51	1.06	21.0	
14.3	0.70	2.24	2.17	1.25	16.5	
14.3	0.66	2.87	2.13	1.46	16.7	
14.5	0.72	2.76	2.08	1.19	15.1	
14.6	0.69	2.66	1.66	1.25	17.2	
14.6	0.71	2.74	1.45	1.04	12.7	
14.9	0.69	2.54	2.08	1.22	16.8	
15.3	0.61	2.67	1.97	1.27	16.3	
3.8[b]	0.84	3.7	2.64	...	...	Lyman and Kuiken (21)
7.3	0.87	2.92	...	...	...	Mitchell *et al.* (23)
7.6	1.19	3.00	...	...	...	Sauberlich *et al.* (29)
8.4	...	...	3.57	...	...	Riesen *et al.* (27)
8.8	...	2.20[c]	...	1.072[c]	...	Hamilton *et al.* (17)
8.9	0.7	...	...	...	...	Greene and Black (16)
9.1	...	2.2	...	...	...	Schweigert (30)
9.2	0.76	2.19	...	...	...	Mitchell *et al.* (23)
9.3	...	2.4	...	...	...	Schweigert (30)
9.4	0.53	3.2	2.13	...	...	Baumgarten *et al.* (5)
11.4	0.99	2.4	...	...	...	Sauberlich *et al.* (29)
13.9	0.48	2.31	1.51,1.44	...	...	Horn *et al.* (19)
7.9-11.9	0.42-0.72[c]	...	...	0.83-1.44[c]	...	Doty *et al.* (11)
9.0-13.0	0.80-1.00	2.79-3.16	1.22-1.61	...	...	Miller *et al.* (22)
9.9-17.8	...	...	...	...	1.25-5 4 [d]	Rodriguez *et al.* (28)
....	0.6[c]	2.5	...	1.1	...	Block and Bolling (7)
....	...	...	...	...	0.79-6.21[d]	Burkholder *et al.* (9)
....	...	...	...	...	1.64-4.2 [d]	Leng *et al.* (20)
....	...	...	...	...	1.53-3.0 [d]	Richey and Dawson (26)
....	...	...	...	...	1.49-3.13[d]	Gorfinkel (15)

[a] Not included in statistical calculations.
[b] Calculated by authors from published data.
[c] Chemical method.
[d] Mg./100 gm. whole corn.

corn were, respectively: 1) tryptophan, 8.9 and 6.9 mg.; 2) lysine, 31.1 and 26.6 mg.; 3) methionine, 20.3 and 18.1 mg.; 4) cystine, 14.6 and 12.8 mg.; 5) nicotinic acid, 0.26 and 0.17 mg. As the protein increases one finds decreases in the percentages of tryptophan, lysine, and nico-

tinic acid associated with the increase. These data do not, however, show marked changes in the percentages of the sulfur amino acids in crude protein as the amount of protein in the corn increases. This may be explained by inadequacies of methodology, or by the fact that the percentages of these amino acids in zein are not much lower than their percentages in whole corn protein.

Student's *t*-test was applied to test the statistical significance of the differences between the means for the groups compared. Data from the statistical calculations are assembled in Table IV. The differences

TABLE IV

QUANTITIES OF FIVE NUTRIENTS IN LOW-PROTEIN AND HIGH-PROTEIN CORN PER 100 G. DRY MATTER

Groups Compared, and Means	Tryptophan		Lysine		Methionine		Cystine		Nicotinic Acid	
	In Corn	In Protein	In Corn	In Protein	In Corn	In Protein	In Corn	In Protein	In Corn	In Protein
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Low-protein, 9.8%	87	888	314	3114	199	2029	144	1463	2.53	25.8
High-protein, 14.3%	99	695	380	2659	239	1815	182	1284	2.40	16.9
Mean difference	12**	193**	66**	455**	40*	214	38**	179	0.13	8.9**

* Significant at 5% level.
** Significant at 1% level.

between the means for tryptophan and lysine, both in corn and in corn protein, and for cystine in corn and nicotinic acid in corn protein are significant at the 1% level; the difference for methionine in corn is significant at the 5% level; the differences for sulfur acids in corn protein and for nicotinic acid in corn are not statistically significant.

Results of assays of these nutrients, as published by other laboratories, are included in Table III. These data from other assayists fall, in general, in the range reported in this investigation. In an abstract released earlier (13) the authors reported that the percentage of nicotinic acid in high-protein corn was less than in low-protein corn. The samples have since been submitted to further study and better values for nicotinic acid are shown in Tables II and III. The range in content of nicotinic acid for the corn samples as reported in this study is not so wide as is shown among the findings reported by others. Unfortunately several investigators who have reported nicotinic acid assays of

corn did not report the protein content. Rodriguez and co-workers (28) attempted to correlate the amounts of protein and nicotinic acid, and they concluded that there was probably some relationship between the two.

Increase in Percentages of Zein with Increases in Crude Protein. Various studies on the nutritive value of corn grain have been concerned with its zein content and its deficiencies in critical amino acids. Osborne and Clapp (24) analyzed this alcohol-soluble protein for 13 amino acids but found no tryptophan and no lysine. Soon after their report of these findings Willcock and Hopkins (32) announced that the addition of tryptophan prolonged the life of mice fed zein rations; without the tryptophan the animals died in a short time. Osborne and Mendel (25) demonstrated that the addition of both tryptophan and lysine to a ration containing zein permitted young rats to grow slowly. Hansen and his co-workers (18) in studies of fractions of crude corn protein showed that the amounts of zein and of total protein in corn are positively correlated. They found, however, that the increase in zein content was accompanied by an increase in the nonzein protein, but the increase in zein was more rapid than the increase in the zein-free fraction. The data reported in this paper are in agreement with these findings.

The increases in yield which frequently accompany the application of nitrogen fertilizers have brought about a rapid expansion in their use; it is accordingly important that data be compiled to show whether changes occur in the nutritive value of the crude protein in the crops harvested. After the assays reported in Tables II, III, and IV were completed, tests were made to find the amount of zein present in each of several other available samples which differed in their content of crude protein as the result of inherited differences or of added nitrogen fertilizer. Table V shows the cultural methods followed and the yields obtained. Table VI shows the percentages of zein and nonzein protein in these samples, and several trends are evident. An increase in the percentage of crude protein in corn, whether it was the result of inherited differences or of nitrogen fertilization, was paralleled by an increase in the percentage of zein in the crude protein, and by a decrease in the ratio of the nonzein protein to zein. For example, Sample No. 3-A contained 7.2% crude protein and 25.2% of the protein was zein, whereas Sample No. 4-B had 11.7% crude protein and 33.2% of the crude protein was zein. Comparison of data for samples 3-A and 3-B shows that 250 lb. of nitrogen instead of 50 lb. of nitrogen per acre caused the following increases in yields of the hybrid Dixie 17 on a per-acre basis: weight of shelled corn, 94.6%; crude protein,

TABLE V

CULTURAL METHODS AND YIELDS OF CORN GRAIN AND OF CORN PROTEIN

Sample No.	Kind of Corn and Portion of Corn Kernel	Nitrogen Fertilizer Treatment per Acre	Plant Population per Acre	Yield of Air-Dry Shelled Corn per Acre	Crude Protein	
					In Moisture-Free Sample	Yield per Acre
		lb.		*lb.*	%	*lb.*
1	C17×K4; whole	Unknown	Unknown	Unknown	8.1	....
2 A	US 13[a]; whole	Unknown	Unknown	Unknown	8.3	....
2 B	US 13; endosperm	Unknown	Unknown	Unknown	7.2	....
	Difference between whole corn and endosperm				− 1.1	
3 A	Dixie 17[b]; whole	50	11,100	3592	7.2	242
3 B	Dixie 17[b]; whole	250	12,600	6989	9.9	651
	Difference	+200	+ 1,500	+3397	+ 2.7	+409
4 A	Ill. high-protein[b]; whole	50	7,900	Unknown	13.7	....
4 B	Ill. high-protein[b]; whole	50	11,990	1614	11.7	179
	Difference	....	+ 4,090	....	− 2.0	....
4 B	Ill. high-protein[b]; whole	50	11,990	1614	11.7	179
4 C	Ill. high-protein[b]; whole	250	10,000	2702	17.6	446
	Difference	+200	− 1,990	+1088	+ 5.9	+267
4 D	Ill. high-protein[b]; whole	120	Unknown	Unknown	17.3	....
4 E	Ill. high-protein[c]; whole	Unknown	Unknown	Unknown	17.7	....
4 F	Ill. high-protein[d]; endosperm	Unknown	Unknown	Unknown	18.7	....
	Difference between whole corn and endosperm				+ 1.0	....

[a] U.S. 13 endosperm furnishes 80.5% of dry matter and 69.5% of the protein.

[b] Samples secured from a cooperative experiment conducted by the Departments of Field Crops and Soils of the Missouri Agricultural Experiment Station.

[c] Ill. High-protein corn secured from the University of Illinois in 1951.

[d] Ill. High-protein endosperm of sample 4E furnishes 81.1% of dry matter and 84.7% of the protein.

TABLE VI

INCREASE IN ZEIN CONTENT WHICH ACCOMPANIES INCREASE IN CRUDE PROTEIN IN CORN

Sample No.	Kind of Corn and Portion of Corn Kernel	Zein			Nonzein Protein		Ratio of Nonzein Protein to Zein
		In Moisture-Free Sample	In Total Protein	Yield per Acre	In Moisture-Free Sample	Yield per Acre	
		%	%	*lb.*	%	*lb.*	
1	C17×K4; whole	2.0	24.6	...	6.1	...	3.06:1
2 A	US13; whole	2.0	24.0	...	6.3	...	3.17:1
2 B	US13; endosperm	2.7	37.8	...	4.5	...	1.65:1
	Difference between whole corn and endosperm	+ .7	+13.8	...	− 1.8	...	−1.52:1
3 A	Dixie 17; whole	1.8	25.2	62	5.4	180	2.97:1
3 B	Dixie 17; whole	3.9	39.3	256	6.0	395	1.54:1
	Difference	+2.1	+14.2	+194	+ .6	+215	−1.43:1
4 A	Ill. high-protein; whole	5.1	37.2	...	8.6	...	1.69:1
4 B	Ill. high-protein; whole	3.9	33.2	59	7.8	120	2.02:1
	Difference	−1.2	+ 4.0	...	− 0.8	...	+0.33:1
4 B	Ill. high-protein; whole	3.9	33.2	59	7.8	120	2.02:1
4 C	Ill. high-protein; whole	8.9	50.5	225	8.7	221	0.98:1
	Difference	+5.0	+17.3	+166	+ 0.9	+101	−1.04:1
4 D	Ill. high-protein; whole	6.8	39.4	...	10.4	...	1.54:1
4 E	Ill. high-protein; whole	6.5	36.8	...	11.2	...	1.71:1
4 F	Ill. high-protein; endosperm	8.5	45.5	...	10.2	...	1.20:1
	Difference between whole corn and endosperm	+2.0	+ 8.7	...	− 1.0		− .51:1

169.0%; zein, 312.9%; and nonzein protein, 119.4%. As these yields increased, the ratio of nonzein protein to zein fell from 2:97:1 to 1:54:1. A similar increase in added nitrogen in the case of Illinois High Protein corn (Samples 4-B and 4-C) resulted in increases in yield on a per-acre basis as follows: weight of shelled corn, 67.4%; crude protein, 149.2%; zein, 281.4%; and nonzein protein, 84.2%. In this case the increase in added nitrogen caused the ratio of nonzein protein to zein to fall from 2:02:1 to 0:98:1. These data are in harmony with findings published recently by Mitchell *et al.* (23) and by Sauberlich *et al.* (29), who reported significant increases in crude protein with fertilization, but who found that the increase was largely in the zein fraction, causing decreases in the percentages of the critical amino acids tryptophan and lysine in the crude protein of the corn of higher nitrogen content. An exception to this opinion was expressed by Miller and his co-workers (22). They interpreted their data on essential amino acids in selected corn hybrids as indicating that the amounts of tryptophan, lysine, and methionine in their samples varied directly with the crude protein content; they considered, however, that the distribution of the amino acids under study was the same in both

low-protein and high-protein corn, and that the quality of the protein in their samples was not changed with increases in the amount of protein within the range from 8.5% to 14.1%. There is great value in the increases in yield which result from nitrogen fertilization, but one must bear in mind that the marked increase in zein which accompanies fertilization means that there is a corresponding decrease in the concentrations of the essential nutrients tryptophan and lysine in the crude protein of the corn from the heavily fertilized fields.

Practical Aspects of Changes in Composition of Crude Protein with Increase of Protein in Corn Grain. Workers interested in the improvement of the nutritional characteristics of corn by breeding methods are in disagreement as to the best procedures to follow. One group assumes that efforts should be directed toward an improvement in amino acid balance with a minimum change in crude protein. The second group assumes that an increase in crude protein would be desirable regardless of its effect on quality.

The protein content from a particular strain or hybrid is limited by its genetic potential, which can be changed materially by the plant breeder. However, whether or not this possible maximum protein content is attained by a given strain or hybrid may be greatly influenced by cultural practices and by climate. Although it is possible to increase the percentage of protein in corn, the success of corn breeders in increasing the essential amino acids has been limited. In other words, an increase in protein content brings an increase in the zein fraction, with only small increases in tryptophan and lysine.

Presumably the protein of high-protein corn is inferior to the protein of low-protein corn in biological value for nonruminants. However that may be, it seems probable that high-protein corn is superior to low-protein corn, if the diet contains no other source of nitrogen. In other words, the total amount of essential amino acids may increase, even though the percentages of the amino acids in the protein may decrease. The biological value of protein is much less important for ruminants than it is for nonruminants. One would expect, then, that an increase in the protein content of corn consumed by these animals would be accompanied by a corresponding decrease in the required amount of protein supplements. If so, high-protein corn would be much superior to low-protein corn in the rations of cattle and sheep.

Yield cannot be neglected as a factor in choosing strains and hybrids for corn production. High-protein corn with a low yield in bushels per acre would be unpopular with producers. If corn breeders could develop a high-yielding strain with a protein content of 15% or better, then the feasibility of producing and utilizing a high-protein

corn might become a matter of practical importance to the food and feed industry.

Literature Cited

1. Anonymous. Improvement of nutrient value of food by plant breeding, guided by chemical control. Nutrition Rev. **7:** 186–187 (1949).
2. Anonymous. Improvement of the nutritive value of corn by plant breeding and selection. Nutrition Rev. **8:** 241–243 (1950).
3. Anonymous. Improving the nutritive value of corn. Nutrition Rev. **10:** 70–72 (1952).
4. Anonymous. Nutritive value of corn. Nutrition Rev. **11:** 50–52 (1953).
5. Baumgarten, W., Mather, Adaline N., and Stone, L. Essential amino acid composition of feed materials. Cereal Chem. **23:** 135–155 (1946).
6. Barton-Wright, E. C. The microbiological assay of the vitamin B-complex and amino acids. Pitman Publishing Corporation: New York (1952).
7. Block, R. J., and Bolling, Diana. Nutritional opportunities with amino acids. J. Am. Diet. Assoc. **20:** 69–76 (1944).
8. Block, R. J., and Bolling, Diana. The amino acid composition of proteins and foods; analytical methods and results. C. C. Thomas: Springfield, Ill. (1951).
9. Burkholder, P. R., McVeigh, Ilda, and Moyer, Dorothy. Niacin in maize. Yale Jour. Biol. Med. **16:** 659–663 (1944).
10. Doty, D. M. Methods for estimation of some amino acids in corn grain. Ind. Eng. Chem. (Anal. Ed.) **13:** 169–172 (1941).
11. Doty, D. M., Bergdoll, M. S., Nash, H. A., and Brunson, A. M. Amino acids in corn grains from several single cross hybrids. Cereal Chem. **23:** 199–209 (1946).
12. Flynn, Laura M., Williams, V. B., O'Dell, B. L., and Hogan, A. G. Medium for assay of vitamins with lactic acid bacteria. Anal. Chem. **23:** 180–185 (1951).
13. Flynn, Laura M., Zuber, M. S., Leweke, D. H., Grainger, R. B., and Hogan, A. G. The variations in concentration of several essential amino acids and of nicotinic acid associated with increase in crude protein in corn. Abstracts of Papers, 118th meeting of American Chemical Society, 23C (Sept. 1950).
14. Folin, O. The preparation of sodium tungstate free from molybdate, together with a simplified process for the preparation of a correct uric acid reagent (and some comments). J. Biol. Chem. **106:** 311–314 (1934).
15. Gorfinkel, L. The influence of crossing on the nicotinic acid content of maize. J. Agr. Sci. **38:** 339–342 (1948).
16. Greene, R. D., and Black, A. The microbiological assay of tryptophane in proteins and foods. J. Biol. Chem. **155:** 1–8 (1944).
17. Hamilton, T. S., Nevens, W. B., and Grindley, H. S. The quantitative determination of amino acids of feeds. J. Biol. Chem. **48:** 249–272 (1921).
18. Hansen, D. W., Brimhall, Bernadine, and Sprague, G. F. Relationship of zein to the total protein in corn. Cereal Chem. **23:** 329–335 (1946).
19. Horn, M. J., Jones, D. B., and Blum, A. E. Methods for microbiological and chemical determinations of essential amino acids in proteins and foods. U. S. Dept. Agr. Misc. Publ. No. 696.7 (1950).
20. Leng, E. R., Curtis, J. J., and Shekleton, M. C. Niacin content of waxy, sugary, and Dent F_2 segregating kernels in corn. Science **111:** 665–666 (1950).
21. Lyman, C. M., and Kuiken, K. A. The amino acid composition of meat and some other foods. I. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Tex. Agr. Expt. Sta. Bull. 708 (1949).
22. Miller, R. C., Aurand, L. W., and Flach, W. R. Amino acids in high and low protein corn. Science **112:** 57–58 (1950).
23. Mitchell, H. H., Hamilton, T. S., and Beadles, Jessie R. The relationship between the protein content of corn and the nutritional value of the protein. J. Nutr. **48:** 461–476 (1952).
24. Osborne, T. B., and Clapp, S. H. Hydrolysis of the proteins of maize. Am. J. Physiol. **20:** 477–493 (1908).

25. Osborne, T. B., and Mendel, L. B. Nutritive properties of proteins of the maize kernel. J. Biol. Chem. **18:** 1–16 (1914).
26. Richey, F. D., and Dawson, R. F. A survey of the possibilities and methods of breeding high-niacin corn. Plant Physiol. **23:** 238–254 (1948).
27. Riesen, W. H., Schweigert, B. S., and Elvehjem, C. A. Microbiological determination of methionine in proteins and foodstuffs. J. Biol. Chem. **165:** 347–358 (1946).
28. Rodriguez, Lorraine D., Hunt, C. H., and Bethke, R. M. The protein, niacin, and pantothenic acid contents of corn inbred lines. Cereal Chem. **27:** 67–70 (1950).
29. Sauberlich, H. E., Wan-Yuin, Chang, and Salmon, W. D. The amino acid and protein content of corn as related to variety and nitrogen fertilization. J. Nutr. **51:** 241–250 (1953).
30. Schweigert, B. S. Amino acid content of foods. J. Am. Diet. Assoc. **24:** 939–944 (1948).
31. Strong, F. M. Report on nicotinic acid. J. Assoc. Offic. Agr. Chem. **30:** 398–412 (1947).
32. Willcock, Edith G., and Hopkins, F. G. The importance of individual amino-acids in metabolism. J. Physiol. **35:** 88–102 (1906).
33. Wood, E. C. The computation of microbiological assays of amino-acids and other growth factors. Anal. **72:** 84–90 (1947).

THE SPECIFIC HEAT OF SOME CEREAL GRAINS[1]

R. W. DISNEY[2]

ABSTRACT

The specific heat of wheat, determined with a modified Bunsen Ice Calorimeter, ranged from 0.582 cal/g/°C at 33.6% moisture content to 0.302 cal/g/°C at 1.4%. Preliminary results on No. 1 Northern Manitoba wheat led to a closer study of laboratory-grown Bersée wheat, with a view to detecting any hysteresis effects between wetted and dried grain. Between 34% and 0% moisture content, on the desorption curve, there seemed to be three breaks making necessary the fitting of four short straight-line regressions to the results. These breaks occurred at about 1.8%, 7.7%, and 23.7% moisture content, and it is suggested that they may be due to some change in the hydration, in the course of the determination, of a substance present in the wheat. No hysteresis effects are apparent, although this may be due to the wetted grain being nonviable. Determinations were made on a few samples of barley and paddy (rough rice), little difference being found between the specific heat of these grains and wheat, over the range of moisture content investigated.

A knowledge of the thermal properties of grain is essential if it is hoped to study the problems encountered in storage of grain in bulk. It is upon these properties that any change of temperature or moisture content will largely depend. This has been realized for some time, and several workers (1, 4, 5) have investigated these properties, with special emphasis on the thermal conductivity.

The specific heat of grain has been determined indirectly by Babbitt (1) from measurements of the thermal conductivity and thermal diffusivity. Pfalzner (6) has measured the specific heat of wheat by a "method of mixtures," and finds that his results are in general agreement with those of Babbitt. Other workers (2, 7, 9) have determined the specific heat of wheat flour, in connection with investigations of its heat of hydration.

The investigations described below had been in hand for some time before Pfalzner's paper was published, and the aim of the work was found to be rather similar to his.

The aim was to obtain a direct measurement of the specific heat of grain and its variation with moisture content. It was also hoped that some light would be thrown onto the condition of the adsorbed water.

Apparatus

The apparatus is shown in diagrammatic form in Fig. 1. It consisted of a slightly modified version of the Bunsen Ice Calorimeter (3).

[1] Manuscript received October 19, 1953.

[2] Department of Scientific and Industrial Research, Pest Infestation Laboratory, London Road, Slough, Bucks, England.

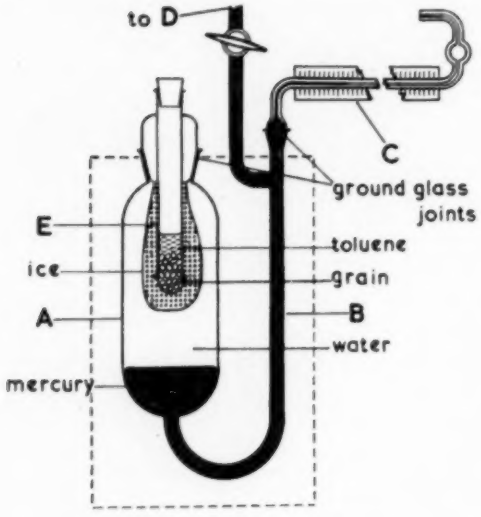


Fig. 1. Diagram of ice calorimeter.

The bulb *A* contained boiled redistilled water and was connected via the tube *B* to the horizontal manometer *C*. The lower part of *A*, the tube *B*, and the manometer *C* contained redistilled mercury. The tube *B* was connected via a tap to an adjustable mercury reservoir *D*. This reservoir and tap allowed the position of the mercury in the manometer to be adjusted. A tube *E* projected into *A*, and ice was formed on the outside of this tube by evaporation of ether inside it.

The part of the apparatus contained inside the broken line in the diagram was immersed in a bath containing a mixture of water and ethylene glycol. The temperature of this bath was controlled by circulating a cooled liquid through a copper coil by means of a pump controlled by a mercury/toluene thermostat. The liquid in the bath was stirred continuously by an electric stirrer, and the temperature was maintained at $+0.1$°C. within fine limits. This temperature ensured that the ice surrounding *E* was continuously melting at a very slow rate, and thus the temperature within the tube *E* was, for all practical purposes, 0.0°C.

Calibration. The apparatus was calibrated electrically by placing a small heating coil of about 100 ohms at the bottom of the tube *E*, and passing a measured current through the coil with a measured potential difference for a measured time. By reading the manometer before and after the passage of the current, and correcting for the

slight continuous melting of the ice, the quantity of heat corresponding to one scale division of the manometer was determined.

The calibration was repeated 20 times and the arithmetic mean of the results was taken to be the true value. This value was checked against the theoretical calibration obtained from the change of volume with change of state from ice to water, the latent heat of fusion of ice (International Critical Tables), and the bore of the manometer tube. The experimental mean was 16.535 cal/in, and the theoretical value was 16.532 cal/in, the difference being well within the limits of experimental error.

Method

The sample of grain whose specific heat was to be determined was placed in a stoppered glass tube and weighed. The stopper was then replaced by another, through which projected a mercury-in-glass thermometer graduated to 0.1°C. The glass tube and its contents were then placed in cotton wool, and the whole left to stand in the room to allow equilibration of grain temperature with the thermometer.

The calorimeter was set up with about 5 ml. of toluene in the calorimeter tube *E* to assist the heat transfer from the grain to the ice. It had previously been verified that there was no measurable evolution of heat on mixing grain and toluene at the same temperature. When the toluene had cooled to 0°C. a steady state was reached in which the ice was slowly melting. The manometer was read and a stop-clock started. Ten minutes later the manometer was again read; the temperature of the grain was read from the thermometer, and a few grams of grain were dropped into the calorimeter from the sample tube. The tube with its remaining grain was weighed immediately afterwards. Fourteen minutes were found to be ample time for the grain in the calorimeter to give up its heat to the ice, and for the steady state to be restored. The manometer was therefore read 14 minutes after the second reading and again after a further 10 minutes. The specific heat was calculated from the relation:

$$C = 16.535 \left[a_2 - \frac{t_2}{2} \left(\frac{a_1}{t_1} + \frac{a_3}{t_3} \right) \right] \Big/ mT$$

where: C = average specific heat of grain between $T°$ and 0°C.;
T = difference between initial and final temperatures of grain in degrees Centigrade (since final temperature is 0°C., T is effectively the initial temperature of the grain in degrees Centigrade);

m = mass of grain dropped into calorimeter;
a_1, a_2, a_3 = differences between first and second, second and third, and third and fourth manometer readings; and
t_1, t_2, t_3 = times elapsing between manometer readings.

The specific heat of each sample was determined on 12 successive portions of the sample, and the arithmetic mean and standard error were calculated.

The main source of error in determining specific heats by the Bunsen Ice Calorimeter is in measuring the quantity of ice melted by reading the change in position of the mercury in the manometer. It is therefore desirable to have the manometer movement as great as possible, and this may be achieved by melting a comparatively large volume of ice, either by using a large quantity of grain or by raising the grain to a high initial temperature. A high grain temperature is undesirable, since its temperature immediately on dropping into the calorimeter would not be accurately known. Room temperature was therefore used to avoid heat gain or loss by the grain in transfer to the calorimeter. If more than about 5 g. of grain were used at any one time, some heat was lost other than to the ice and this resulted in a low value for the specific heat. Between 3 and 4 g. of grain were therefore generally used in determinations.

Moisture contents were determined by drying duplicate ground samples in a ventilated oven at 113°C. for 4 hours, and all results were calculated as a percentage of the wet weight. This method is standard in this Laboratory, and gives results which compare very closely with those obtained using a temperature of 130°C. for 1 hour. Results below 2% are reported to two places of decimals.

A preliminary series of determinations was carried out on a batch of No. 1 Northern Manitoba wheat with an initial moisture content of 13.7%. Samples from this batch were conditioned to other moisture contents by drying in a ventilated oven at temperatures up to 60°C., or by addition of liquid water to grain contained in air-tight jars. Very dry samples of grain were obtained by drying overnight in a ventilated oven at 113°C., and also by drying over phosphorus pentoxide for several weeks in a vacuum desiccator.

A more detailed study was made of Bersée wheat grown at the Laboratory. This is a soft white wheat of French origin, extensively grown as a high-yielding winter or spring variety. Small samples were harvested at intervals before the main harvest, and kept in sealed jars in a refrigerator until required for use. A sample of the main harvest was kept and about 1 kg. was placed in a ventilated oven and slowly

TABLE I

THE SPECIFIC HEAT OF WHEAT, MALTING BARLEY, AND SWAMP PADDY AT VARIOUS MOISTURE CONTENTS

Sample No.	Moisture Content (Wet Wt. Basis)	Specific Heat cal/g/°C (Mean)	No. of Replicates	Standard Error	Type of Conditioning[a]	Germination
	%					%
Manitoba wheat						
1	17.5	0.447	9	0.0027	D	...
2	15.3	0.416	12	0.0021	D	...
3	13.7	0.398	13	0.0020	A	...
4	10.1	0.367	13	0.0039	C	...
5	4.9	0.333	12	0.0024	C	...
6	1.89	0.318	12	0.0024	S	60
7	1.29	0.310	12	0.0029	S	...
Bersée wheat			**Desorption**			
8	33.6	0.582	10	0.0016	S	100
9	29.5	0.549	12	0.0019	B	100
10	25.8	0.525	12	0.0027	B	100
11	22.3	0.491	10	0.0020	B	100
12	19.9	0.476	14	0.0034	B	100
13	16.2	0.429	12	0.0023	B	100
14	13.7	0.405	12	0.0014	A	100
15	8.3	0.350	12	0.0026	C	100
16	6.1	0.334	11	0.0021	C	100
17	4.2	0.322	11	0.0016	C	100
18	2.8	0.313	12	0.0014	C	100
19	2.1	0.311	12	0.0017	C	100
20[b]	1.48	0.305	12	0.0033	S	74
21	1.40	0.302	12	0.0018	C	0
22	0.87	0.303	11	0.0025	C	0
23	0.14	0.307	11	0.0027	C	0
			Adsorption			
24[c]	1.99	0.306	12	0.0023	D	0
25[c]	3.2	0.313	10	0.0020	D	0
26[c]	4.4	0.326	11	0.0023	D	0
27[c]	9.8	0.368	12	0.0018	D	0
28[c]	18.2	0.457	12	0.0026	D	0
Malting barley						
29	16.5	0.436	11	0.0026	D	...
30	13.8	0.387	10	0.0026	A	100
31	9.4	0.359	11	0.0016	E	...
Swamp paddy (Gold Coast)						
32	14.9	0.417	10	0.0037	D	...
33	11.5	0.377	11	0.0032	A	41
34	9.2	0.358	10	0.0040	E	39

[a] Key to types of conditioning used:

A. No conditioning. Grain as supplied or as harvested from field.
B. No conditioning. Grain samples harvested early from field on different days.
C. Heated in ventilated oven over period of weeks. Samples taken at various suitable moisture contents.
D. Wetted by addition of water or water vapor.
E. Dried in vacuum at 30°C.
S. Conditioning of special samples, Nos.:

6. Dried in vacuum over phosphorus pentoxide at room temperature. Started from sample 5.
7. Heated overnight in ventilated oven at 113°C. Started from sample 5.
8. Started from sample 14. Soaked in excess distilled water for 48 hours, and then gently dried in room to required moisture content.
20. Dried from sample 14 in vacuum. Maximum temperature reached, 80°C.

[b] Not included in regression line.
[c] Started from sample 23.

dried at gradually increasing temperatures. Samples were taken from the oven at intervals and placed in sealed jars, and in this way samples were obtained at decreasing moisture content to 0.14%, the final oven temperature being 110°C. One sample from the main harvest was dried in a vacuum oven to 1.48% moisture content, the maximum temperature used being 80°C. In order to discover any possible hysteresis effects, some of the wheat which had been dried to 0.14% was allowed to regain moisture from the air, samples being removed up to 4.4% moisture content, and two samples were wetted with water to 9.8% and 18.2% moisture content respectively.

Some samples of barley and paddy (rough rice) were conditioned in the way described above, but determinations were made at only a few moisture contents, to obtain a comparison with the wheat.

Germination tests were carried out on some of the samples, using 9-cm. Petri dishes containing 80 g. of sterilized sand or a standard seed test pad soaked in distilled water. Any grain which had not germinated in 14 days at room temperature was taken to be nonviable.

Results and Discussion

The results which were obtained are summarized in Table I.

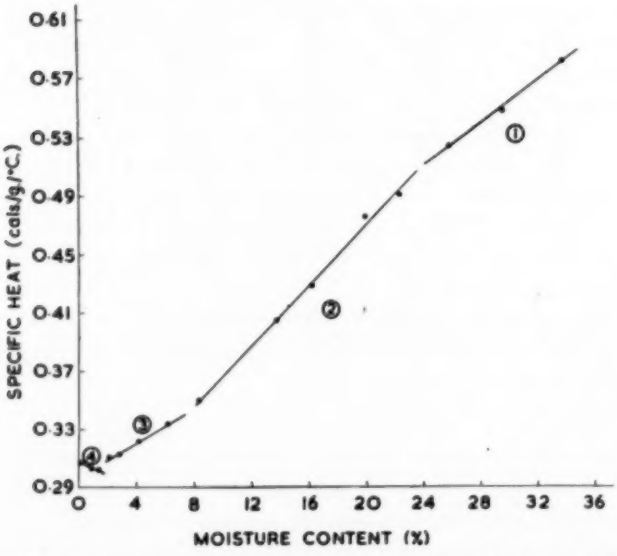


Fig. 2. Relation between specific heat and moisture content of Bersée wheat during desorption. The numbers in circles refer to the numbering of the regression lines in Table II.

The first graph (Fig. 2) shows the values of specific heat at various moisture contents, obtained during natural and artificial drying of Bersée wheat (i.e., during desorption). The set of four regression lines shown were determined by the method of least squares, and were chosen as they have the minimum total variance for any set of three or four regression lines through the experimental points.

Table II shows the four regression equations with the range of moisture content covered, the residual variance of the equations, and the apparent specific heat of the adsorped water obtained for each line by solving the equation for C when M (moisture content) equals 100%.

TABLE II

REGRESSION EQUATIONS FOR COMPUTING THE SPECIFIC HEAT OF BERSÉE WHEAT AT DIFFERENT MOISTURE LEVELS

Line	Moisture Content Range	Regression Equation[a]	Residual Variance	Apparent Specific Heat of Water[b] (cal/g/°C)
1	23.7 – 33.6%	$C = 0.00731M + 0.336$	6.0×10^{-6}	1.07
2	7.7 – 23.7%	$C = 0.01036M + 0.263$	1.8×10^{-5}	1.30
3	1.8 – 7.7%	$C = 0.00591M + 0.297$	1.0×10^{-6}	0.89
4	0.0 – 1.8%	$C = 0.00406M + 0.307$	1.0×10^{-6}	−0.10

[a] C = specific heat; M = moisture content.
[b] Computed from the regression equation by writing M = 100%.

The desorption curve between 34% and 0% falls into four parts with breaks at about 23.7%, 7.7%, and 1.8% moisture content.

In line 1 the apparent specific heat of the bound water does not differ significantly from unity, or in other words the water seems to be exerting its normal specific heat. In line 2, however, the apparent specific heat of 1.30 is undoubtedly significant. This high result may be due to the exothermic hydration of some substance in the wheat, during cooling in the calorimeter from room temperature, i.e. about 18°C., to 0°C. If this is the case, the lower result for line 1 may be due to all the available hydratable substance being already hydrated at these higher moisture contents. The result in line 3 may be nearer the actual specific heat of the bound water, there being insufficient moisture present for full hydration of any substances. This agrees in part with the observations of Shipley, Campbell, and Maass (8), who suggest that the specific heat of water adsorbed in cellulose may be composed of two parts, one from the heat content of the bound water and the other from the change of binding energy between the water and the adsorbing material.

If wheat is dried below 2% moisture content without being killed (samples 6 and 20), it appears to exert a higher specific heat than the nonviable wheat at a similar moisture content. Although the data available are not conclusive, it may be that when the wheat is killed some substance or substances vital to life are changed chemically, releasing water which then becomes physically adsorped in the grain. This may well be expected to lead to a change in the specific heat. In line 4 the apparent specific heat of water is very low indeed, but since the observations extend over a very short moisture content range, the negative value obtained is probably not significantly different from a near-zero value. It is to be expected that a very low specific heat for water would occur in this region, where presumably all the water is held in monomolecular films. Even a negative specific heat for water may possibly occur if it be supposed that the effect of a monomolecular film of water in reducing the mobility of the surface molecules of the solid substrate reduces their ability to absorb thermal energy by an amount greater than the increase due to the water itself.

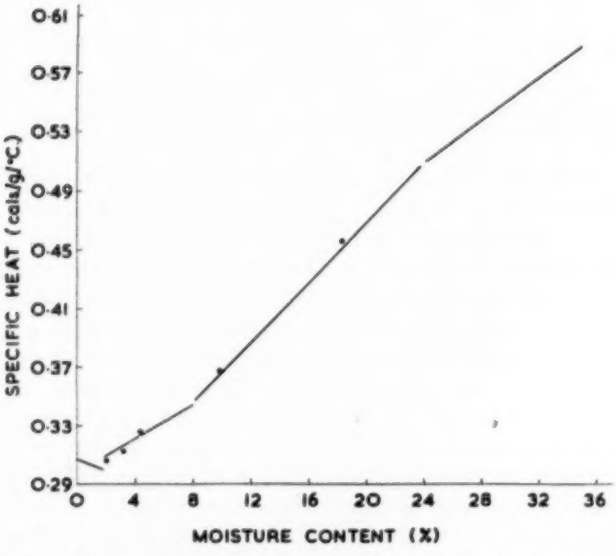


Fig. 3. Points showing the relation between specific heat and moisture content of Bersée wheat during adsorption, compared with regression lines from Fig. 2 which show the same relation during desorption.

Figure 3 shows the regression lines for the desorption of Bersée wheat (from Fig. 2), together with the values obtained for the specific

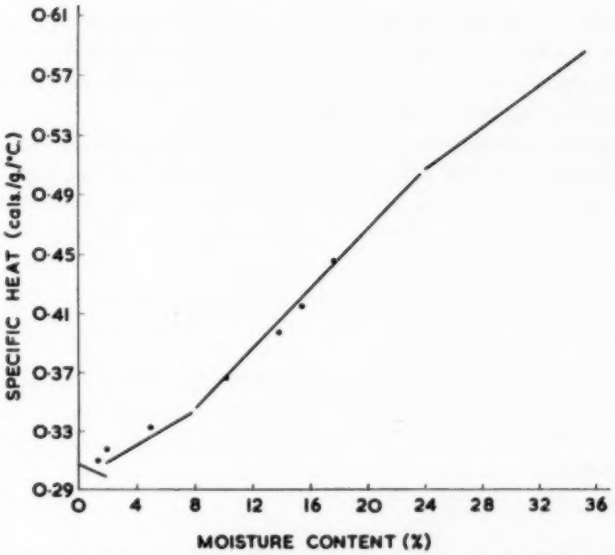


Fig. 4. Points showing the relation between specific heat and moisture content of Manitoba wheat (some points adsorption, others desorption), compared with the regression lines from Fig. 2 which show the same relation for Bersée wheat.

TABLE III

SUMMARY OF DATA BY PREVIOUS WORKERS COMPARED WITH SOME DATA FROM THE PRESENT PAPER

Name and Date	Specific Heat at a Stated Moisture Content			Remarks
	0%	8.3%	13%	
Daniels *et al.* (1920)	0.34[a]		0.43	Wheat flour
Winkler and Geddes (1931)	0.397[a]			Wheat flour
Babbitt (1945)	0.31[a]	0.37		Wheat
Schrenk *et al.* (1949)	0.45			Wheat flour
	0.43			
Pfalzner (1951)	0.283[b,c]	0.343[b,c]	0.377[b,c]	Wheat adsorbing water vapor after initial drying
	0.301[b,c]	0.362[b,c]	0.396[b,c]	
	0.288[b]	0.357[b]	0.396[b]	Wheat wetted with liquid water after initial drying
Moote (1953)	0.314[b]	0.404[b]		Wheat "lot 1"[d]
	0.310[b]	0.407[b]	0.463[b]	Wheat "lot 2"[d]
Disney (1954)	0.307[b]	0.349[b]	0.398[b]	Desorption of Bersée wheat

[a] Calculated from a determination at a higher moisture content on the assumption that the specific heat of adsorbed water is identical with that of free water.
[b] Calculated from the author's stated regression lines.
[c] These two samples were apparently identical in kind and treatment.
[d] The data obtained by Moote (1953) are probably subject to considerable error at high moisture contents, as the method of determination induced some movement of water in the temperature gradient.

heat of the dry sample (0.14%) after it had been wetted to a range of moisture contents (i.e., during adsorption). These points, showing no hysteresis effects, follow the desorption lines fairly closely in spite of the grain having been killed by heat in drying.

Figure 4 shows the results obtained for Manitoba wheat (Canadian hard red spring wheat) compared with the desorption regression lines for Bersée wheat. The graph shows that there is very little difference in the specific heats of the two varieties of wheat over the range of moisture content investigated.

Table III shows a comparison at various moisture contents of the results obtained by previous workers with the results given in this paper.

The data given in the present paper are, by contrast with those of the literature cited, the only ones in which each result is the mean of a series of determinations, thus making possible the calculation of a standard error.

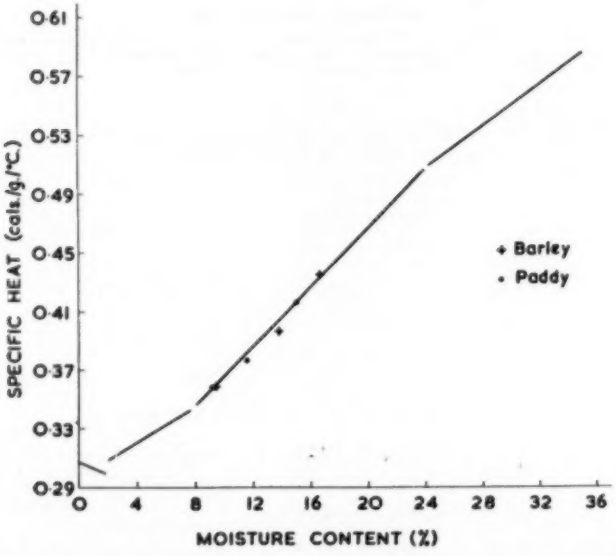


Fig. 5. Points showing the relation between specific heat of two heavily husked grains, barley and paddy (rough rice) and moisture content, compared with the regression lines from Fig. 2 which show the same relation for Bersée wheat.

Figure 5 shows the results of determinations on the other grains studied, and again the desorption regression lines of the Bersée wheat (Fig. 2) are included to allow comparison between these grains and

the wheat. It will be seen that there is little difference between the values obtained for these other grains, and those for wheat.

Acknowledgments

This work formed a part of the program of research of the Pest Infestation Laboratory, and is published by permission of the Department of Scientific and Industrial Research. The author wishes to thank Mr. T. A. Oxley, under whose direction the work was carried out, for his constructive criticism and assistance throughout.

Literature Cited

1. Babbitt, J. D. Thermal properties of wheat in bulk. Can. J. Research **F23:** 388–401 (1945).
2. Daniels, F., Kepner, B. H., and Murdick, P. P. The heat of hydration and specific heat of wheat flour. J. Ind. Eng. Chem. **12 (8):** 760–763 (1920).
3. Glazebrook, R. (Ed.). A dictionary of applied physics. Vol. 1, pp. 49, 563. Macmillan: New York (1922).
4. Moote, Irene. The effect of moisture on the thermal properties of wheat. Can. J. Tech. **31:** 57–69 (1953).
5. Oxley, T. A. The properties of grain in bulk. III. The thermal conductivity of wheat, maize, and oats. J. Soc. Chem. Ind. **63:** 53–55 (1944).
6. Pfalzner, P. M. The specific heat of wheat. Can. J. Tech. **29:** 261–268 (1951).
7. Schrenk, W. G., Andrews, A. C., and King, H. H. Heat of hydration of certain wheat flours and gluten. Cereal Chem. **26:** 51–59 (1949).
8. Shipley, J. H., Campbell, W. B., and Maass, O. The heat content of water adsorbed on cellulose. Can. J. Res. **B17:** 40–50 (1939).
9. Winkler, C. A., and Geddes, W. F. The heat of hydration of wheat flour and certain starches including wheat, rice, and potato. Cereal Chem. **8:** 455–475 (1931).

EXTENSOGRAPH STUDIES OF THE IMPROVING ACTION OF OXYGEN IN DOUGH[1]

C. J. Dempster, I. Hlynka, and J. A. Anderson

ABSTRACT

The extensograph technic previously described has been used to investigate the influence of oxygen on structural relaxation in unleavened dough. Experimentally, doughs were mixed for 3 minutes in atmospheres containing 0, 20, 40, 60, 80, and 100% oxygen.

The structural relaxation curves indicate that oxygen has two distinct effects in dough. Oxygen in doughs of zero reaction time has an initial or immediate effect. The steady state load at the limit of relaxation increases proportionately with concentration up to a limit between 60 and 80% oxygen. In addition oxygen has a time-dependent effect which increases with concentration. At low oxygen concentrations, the rate of structural relaxation increases with reaction time but as the oxygen concentration is increased, this tendency is reversed. The relaxation curves tend to suggest that the reaction of oxygen may proceed to completion at the higher concentrations.

The rate of reaction of oxygen in dough, for each concentration of oxygen, is assessed from measurements of the effect of reaction time on both the relaxation rate constant and the steady state load at the limit of relaxation. The reaction of oxygen is found to be zero order with respect to time and its rate is apparently directly proportional to the oxygen concentration in the mixing atmosphere.

It has been known for many years that oxygen functions as a flour improver. Kent-Jones and Amos (6) reported that the natural improvement in the baking strength of flour on aging was associated with the reaction of atmospheric oxygen. Later Baker and Mize (1) observed the effects on dough and bread properties of mixing doughs in vacuum, in inert gases, and in air and oxygen. With prolonged mixing in the absence of oxygen, no changes were observed in the baking characteristics of the dough. Mixing in air or oxygen for normal times produced improved bread, but prolonged mixing produced detrimental effects. The effect of oxygen was reported to occur only during mixing. Freilich and Frey (5), in studies made with the farinograph, found that oxygen was an important factor in dough development. The effect appeared to be immediate, occurring during the first few minutes of mixing. However, more recent results by Smith and Andrews (8) suggested that oxygen in addition to its immediate effect also had a time-dependent effect in dough. The magnitude of the effect of atmospheric oxygen on dough properties has also been indicated qualitatively in an earlier publication from this laboratory (4);

[1]Manuscript received January 4, 1934. Presented at the 36th Annual Conference of the Chemical Institute of Canada, Windsor, Ontario, June 4-6, 1933. Paper No. 130 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 311 of the Associate Committee on Grain Research (Canada).

it was emphasized that when studying the reaction of potassium bromate the complicating effects of oxygen should be eliminated by mixing in nitrogen.

A renewed interest in the improving action of oxygen in dough has been occasioned by the development of the aeration or batter process of breadmaking patented in Great Britain in 1950 (7). Improvement in loaf characteristics similar to that produced by the addition of customary flour improvers is produced from dough prepared from a batter the ingredients of which are "mixed together at a speed approximating 350 r.p.m., air being incorporated into it at the same time by beating or whisking or other means."

In this laboratory a Brabender Extensograph is used to observe the changes that improvers produce in the structural relaxation of dough (3, 4). Structural relaxation is a term used to designate the relatively slow changes that take place in the physical properties of resting dough subsequent to mixing or mechanical manipulation. The effect of oxygen on structural relaxation has now been investigated in detail by the previously described technic, and the results are presented in this paper.

Materials and Methods

The flour used in this study was milled from Canadian hard red spring wheat grading No. 1 Northern in a Buhler laboratory mill to an extraction of about 70%. The protein content of the flour was 12.2%.

The flour samples were equilibrated in an atmosphere of nitrogen in an attempt to remove any adsorbed oxygen from the flour to ensure that subsequent changes in dough properties resulted only from oxygen incorporated by mixing. Weighed flour samples in separate beakers were placed in a large desiccator which was evacuated to about 1.5 cm. of mercury by operating the Megavac pump for 2–2.5 minutes. The pressure in the desiccator was then restored to atmospheric by admitting nitrogen. The samples were kept in nitrogen overnight before being mixed.

The nitrogen used in these experiments was commercial tank nitrogen, containing at least 99.7% nitrogen. Exploratory experiments, using nitrogen scrubbed through alkaline pyrogallol, flour samples given a rigorous equilibration in such scrubbed nitrogen, and solutions of reagents made from freshly boiled water, showed entirely negligible deviation from the results of experiments using tank nitrogen. Accordingly, it was considered unnecessary to remove the traces

of oxygen from the nitrogen used in the overnight equilibration of flour samples and for the experiments in which doughs were mixed in nitrogen.

Doughs for testing in the extensograph were made with 200 g. flour (14% moisture), 60% water, and 1% sodium chloride (flour basis). The temperature of the dough ingredients was controlled so that the doughs were at 30°C. when taken from the mixer.

A specially designed dough mixer (4) was employed for the experiments in which doughs were mixed in atmospheres of varying oxygen concentration. The mixer is equipped with a bowl which makes an air-tight seal with the body of the mixer so that the mixing chamber can be evacuated. Three connections from the upper part of the mixing chamber lead 1) to a manometer, 2) through a two-way stopcock to a Megavac vacuum pump, and 3) to a separatory funnel by means of which the doughing liquid is added.

The load-extension curves (extensograms) were analyzed by reading the load supported by each dough at a constant sample deformation corresponding to a corrected kymograph extension of 11 cm. A correction was necessary to compensate for the downward displacement of the dough support during the stretching process. Accordingly a small increment was added to the 11-cm. value to give the corrected kymograph extension at which the load is read. The correction, which is linear, is 0 cm. at zero load and 1.44 cm. at 1000-g. load. The structural relaxation curve was obtained by plotting the extensogram load, at this constant sample deformation, against rest period.

Results and Discussion

The effect of oxygen on structural relaxation in dough has been investigated in experiments on doughs mixed in atmospheres containing 0, 20, 40, 60, 80, and 100% oxygen. Doughs prepared in each of these atmospheres were allowed reaction times of 0, 1, 2, 3, and 4 hours between the mixing and shaping operations. Illustrative structural relaxation curves for these doughs, shown in Fig. 1, indicate that the incorporation of oxygen produces marked changes in the physical properties of dough.

The curves of the upper left-hand section of Fig. 1 show the effect of increasing reaction time on structural relaxation in doughs prepared in the absence of oxygen. The behavior of these doughs as represented in these curves serves as a basis of comparison in determining the effect of oxygen upon structural relaxation in dough. With increasing rest period after shaping, the load supported by doughs in

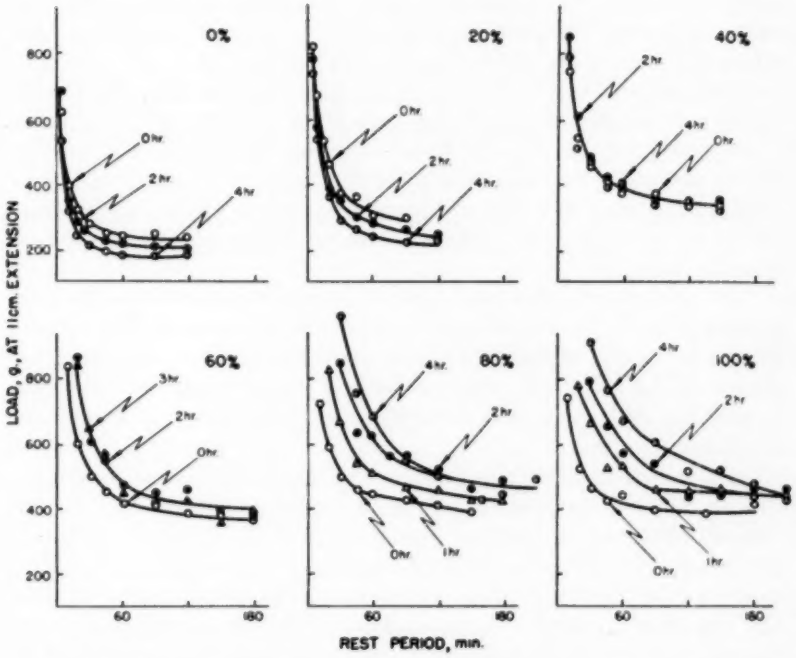


Fig. 1. Structural relaxation curves for doughs mixed in atmospheres of 0, 20, 40, 60, 80, and 100% oxygen.

the extensograph test decreases rapidly at first and then generally reaches a limit designated as the steady state load. (It is to be noted, however, that in some of the cases illustrated in Fig. 1 the load does not reach a limit; rather it appears to decrease linearly with further rest period.) In the absence of oxygen, the structural relaxation curves fall progressively lower on the load axis as reaction time is increased.

The incorporation of oxygen in dough by mixing produces changes in structural relaxation which may be classed, on the one hand, as a time-dependent effect observed previously (3, 4) with bromate and, on the other, as an initial or immediate effect. These will be briefly discussed in this order.

Increasing amounts of oxygen prevent and even reverse the trend shown by the relaxation curves of untreated doughs to undergo a downward displacement with increasing reaction time. With a concentration of 20% oxygen in the atmosphere in the mixer, the relaxation curves still exhibit a downward trend. At a concentration of 40%

oxygen, this downward trend has been eliminated and the relaxation curves superpose. For concentrations of oxygen of 60% and over, the relaxation curves are displaced upwards on the load axis with increasing reaction time. There is a suggestion that the time-dependent reaction of oxygen in dough may proceed to completion. For a level of 60% oxygen, the 2- and 3-hour reaction time relaxation curves nearly superpose. For both 80 and 100% oxygen, the changes in dough properties during the 2-hour reaction time from 2 hours to 4 hours are by no means as great as those occurring during the first 2 hours of reaction time.

The incorporation of increasing amounts of oxygen in dough progressively raises the general level of the 0 hour reaction time relaxation curve on the load axis. Oxygen, therefore, has an initial effect in dough in the minimum reaction time of about 6 minutes required to mix the dough, to scale the 150-g. test piece, and to perform the shaping operations.

A more generalized description of the reaction of oxygen in dough can be obtained by analyzing these structural relaxation data in the manner described in previous publications (3, 4). Changes produced in physical properties by chemical reaction in dough are followed in two ways. In the first of these, rate constants for structural relaxation are calculated, assuming as an approximation that relaxation is exponential. The rate constant characterizes the behavior of the dough at each reaction time. The rate of change of the relaxation rate constant provides a measure of the rate of change of dough properties which is in effect a measure of the rate of the chemical reaction of oxygen in the dough. The second method utilizes the steady state load at the limit of the relaxation curve. The steady state load is regarded as an equilibrium value characteristic of the properties of the completely relaxed dough. Changes in the steady state load are produced by chemical reaction and the rate of change of steady state load is taken as a measure of the rate of chemical reaction.

It may be well to point out here that the analysis of the present structural relaxation data does not yield results of as high a degree of precision as might be desired. Though it is of interest to analyze these data in this way, the results are to be viewed only qualitatively.

Figure 2 shows the changes that take place with reaction time in the structural relaxation rate constants of doughs mixed in atmospheres of varying oxygen concentration. For high levels of oxygen, the relaxation rate constant appears to change linearly with time. A linear relation as indicated by the dashed lines has also been assumed for the data for both 0 and 20% oxygen in spite of the considerable

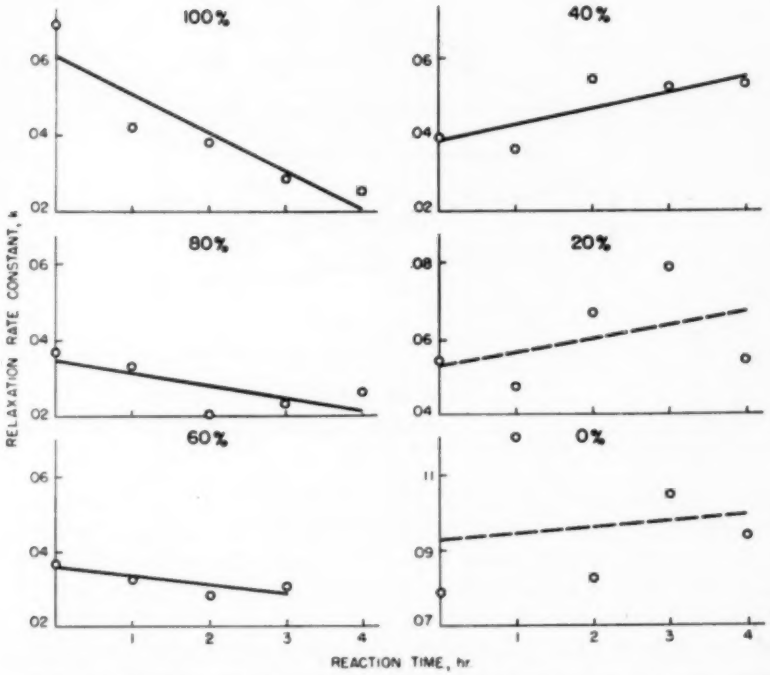


Fig. 2. Effect of reaction time on structural relaxation rate constants for doughs mixed in atmospheres of varying oxygen concentration.

scatter that is shown. The curves are the calculated regression lines for these data.

Varying the oxygen concentration in dough produces a gradual change in the relaxation rate-reaction time relation. At high oxygen concentrations the curves are of negative slope but the slope decreases and tends to become positive as the oxygen level is lowered. The slope of any of these curves is regarded as an approximate measure of the relative rate of reaction of that particular concentration of oxygen in the dough. From the data of Fig. 2, it is therefore possible to derive information about the effect of concentration on the reaction of oxygen in dough. This will be discussed in more detail later.

Figure 3 shows the changes that take place in the steady state load as the oxygen concentration in the mixing atmosphere is altered. In cases where the relaxation curves of Fig. 1 did not appear to reach a stable limit, the values plotted are the loads at which the relaxation curves were judged to change from an exponential decay to a linear

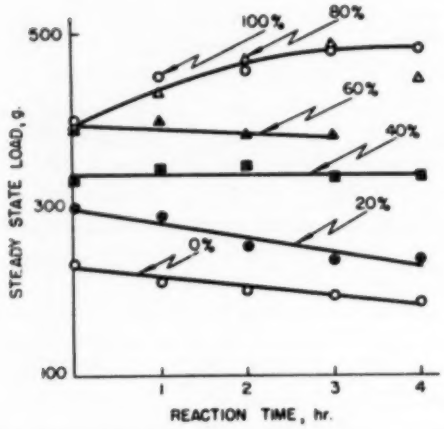


Fig. 3. Effect of reaction time upon steady state or final load supported by doughs mixed in atmospheres of varying oxygen concentration.

decay. For oxygen levels up to 60%, the steady state load appears to change linearly with time, the slope of these curves tending to become positive as the oxygen concentration increases. For oxygen levels of 80% and 100%, the data nearly superpose. Moreover, at these oxygen levels, the steady state load appears to increase linearly to a limit. It may be that, at these concentrations, some constituent of the dough other than oxygen becomes the limiting factor in the reaction; on the completion of the reaction of this material with oxygen, the curves level off.

The slope of any of the curves of Fig. 3 is taken as an approximate measure of the relative rate of the reaction of that particular oxygen concentration. Thus the study of the effect of oxygen upon structural relaxation in dough permits the rate of the reaction of oxygen in dough to be assessed in two ways. These methods of calculating the rate of reaction of oxygen in dough lead to values which, with increasing oxygen concentration, change from positive to negative (Fig. 2) or vice-versa (Fig. 3). It is pointed out that this change in sign is arbitrary and irrelevant to the progressive changes in the reaction rates.

Figure 4 is a plot of the rate of the reaction of oxygen in dough, as assessed from rate constant data (open circles, solid line) and from steady state load values (closed circles, broken line) against oxygen concentration. The ordinates of this figure, because of the method of calculation, are not expressible in customary units of reaction rate but are in the arbitrary units of the slopes of the curves of the two previous

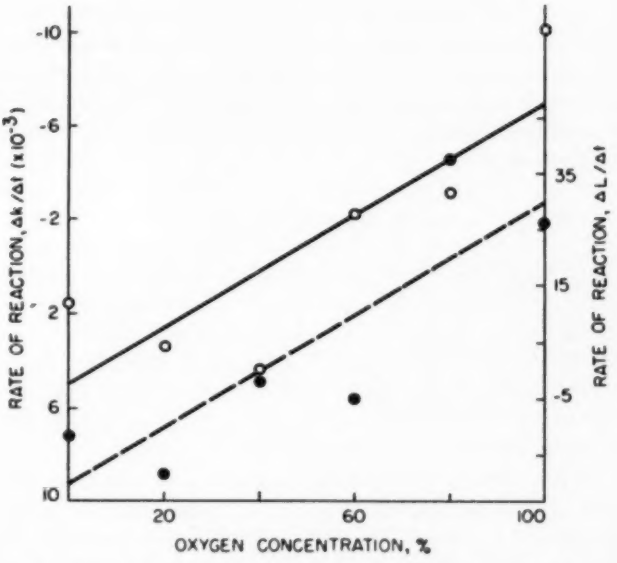


Fig. 4. Comparison of the effect of oxygen concentration on its rate of reaction in dough calculated from (a) the rate of change of structural relaxation rate constants, $\Delta k/\Delta t$ (open circles, solid line) and (b) the rate of change of steady state load, $\Delta L/\Delta t$ (closed circles, broken line).

figures. The scales along the y-axis have been adjusted so that the two sets of data fall in the same range. Assuming a linear relation, regression lines were calculated for the two sets of data. There is a wide scatter of points, but the data suggest that the rate of the reaction of oxygen by both methods varies directly with concentration.

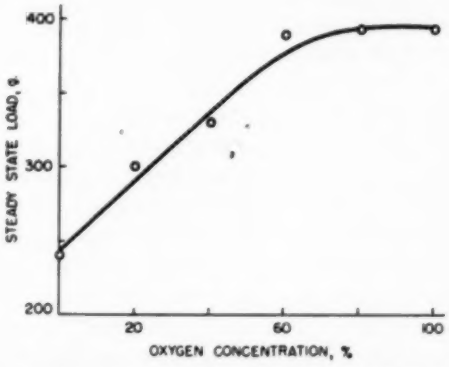


Fig. 5. Relation between oxygen concentration and the initial effect of oxygen in dough as measured by the steady state load of the 0 reaction time relaxation curves.

The steady state load at the limit of the relaxation curves provides additional information about the initial effect of oxygen in dough. Figure 5 shows a plot of the steady state load of the 0 reaction time relaxation curves against oxygen concentration. The initial effect of oxygen increases linearly with concentration up to a limit between 60 and 80% oxygen.

General Discussion

It has been known for many years that oxygen does function as an active improver in breadmaking. However, a clear distinction between the mixing itself and the chemical effect of the reaction of oxygen incorporated by the mixing action has not always been appreciated. The present study of the effect of oxygen upon structural relaxation in dough yields information about the improving action of oxygen under conditions where the mixing effect is constant.

Oxygen has two distinct effects in dough – an initial or immediate effect and a time-dependent effect. The initial effect of oxygen has been recognized for quite some time (1), but it is only recently that the time-dependent effect has been mentioned in the literature (2, 8). The magnitude of both effects depends upon the concentration of oxygen in the atmosphere in which the dough is mixed and therefore upon the actual oxygen concentration in the dough.

It is interesting to compare the results of this study of the effect of oxygen in dough with our previously published results of the effect of another improver, bromate. Oxygen has a marked initial or immediate effect in dough, while bromate, in the concentrations used in our studies, had a negligible effect. However, unpublished results obtained in this laboratory indicate that bromate, in concentrations far exceeding those employed in the baking process, may have an appreciable initial effect. Smith and Andrews (8), in experiments using up to 333 ppm. bromate, have also shown this to be the case.

Both oxygen and bromate exhibit time-dependent effects in dough. The reaction of each appears to be zero order with respect to time. However, with oxygen the results suggest the possibility that at the higher concentrations some dough constituent other than oxygen becomes the limiting factor in the reaction. On the other hand, bromate, even in concentrations which produced changes in dough properties of a greater magnitude, always appeared to be the limiting factor. The time-dependent reactions of both oxygen and bromate appeared to be first order with respect to concentration.

These differences between the reaction of oxygen and bromate in

dough indicate that it may be difficult to explain the reaction in dough of various improving agents by assuming one and the same reaction mechanism. However, from a practical point of view, the exact reaction mechanism is not of prime importance. More important is an appreciation of the role that structural relaxation plays in determining dough behavior. It seems reasonable to suppose that, for any given flour, optimum dough properties for bread production are associated with a particular value of the relaxation rate constant.

In studies of the various factors which can contribute to the production of the optimum structural relaxation rate in dough, the influence of oxygen as an improver must not be overlooked. Generally, the effect of oxygen is not predominant, but it may become significant as in the natural aging of flour, in assessing dough development in the mixer, and in instances where prolonged or rapid mixing is employed in the commercial manufacture of bread.

Literature Cited

1. Baker, J. C., and Mize, M. D. Mixing doughs in vacuum and in the presence of various gases. Cereal Chem. **14:** 721–734 (1937).
2. Dempster, C. J. The effect of potassium bromate on physical properties and structure of flour doughs. Ph.D. thesis, McGill University, 1951.
3. Dempster, C. J., Hlynka, I., and Winkler, C. A. Quantitative extensograph studies of relaxation of internal stresses in non-fermenting bromated and unbromated doughs. Cereal Chem. **29:** 39–53 (1952).
4. Dempster, C. J., Hlynka, I., and Anderson, J. A. Extensograph studies of structural relaxation in bromated and unbromated doughs mixed in nitrogen. Cereal Chem. **30:** 492–503 (1953).
5. Freilich, J., and Frey, C. N. Dough oxidation and mixing studies. VII. The role of oxygen in dough mixing. Cereal Chem. **24:** 436–448 (1947).
6. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry (4th ed.), p. 251. Northern Publ. Co., Ltd.: Liverpool (1947).
7. Rank, Joseph, Ltd., and Hay, James Gordon. Improved method of making bread with untreated and unbleached flour. British Patent No. 646311, Nov. 22, 1950.
8. Smith, D. E., and Andrews, J. S. Effect of oxidizing agents upon dough extensograms. Cereal Chem. **29:** 1–17 (1952).

NIACIN RELATIONSHIPS IN DEVELOPING AND MATURE MAIZE ENDOSPERMS OF BRITTLE AND RELATED GENOTYPES[1]

H. J. Teas,[2] Anna N. Teas,[2] and J. W. Cameron

ABSTRACT

The niacin content of developing maize endosperms of two mutant genotypes, brittle-1 (bt_1) and brittle-2 (bt_2), and their normal counterparts was determined at intervals between 14 and 46 days after pollination. From mid-development on, niacin was higher in both bt_1 and bt_2 than in the normal endosperms. The differences were large on a "per-endosperm" basis and usually larger on a weight basis. The behavior of niacin in the two brittle genotypes was similar to that which has been found for sugary (su_1) maize endosperms.

Mature endosperms homozygous for su_1 and bt_1 together were compared with related ones of normal, bt_1, and su_1. On a per-endosperm basis the su_1 bt_1 type was no higher in niacin than was su_1 from the same ear, but on a weight basis it was twice as high.

Differences in niacin content in maize kernels of various genetic types have been demonstrated by several studies. Mature kernels homozygous for any one of the genes sugary-1 (su_1), dull (du), waxy (wx), brittle-1 (bt_1), brittle-2 (bt_2), shrunken-1 (sh_1) or shrunken-2 (sh_2) are usually slightly to considerably higher in niacin than are normal types (see Teas and Newton (11), Richey and Dawson (8), and references therein).

In comparisons of developing endosperms of two series of su_1 and related normal kernel types, Teas, Cameron, and Newton (10) found that niacin became higher in su_1 at about mid-development and remained higher thereafter. Teas (9) has reported that at maturity the higher niacin in su_1 kernels is largely localized in the aleurone layer of the endosperm.

The present study is concerned with the behavior of niacin in developing endosperms of bt_1, bt_2, and their normal counterparts, and with the interaction of bt_1 with su_1 in mature endosperms. A related paper (4) describes the effects of bt_1 and bt_2 on carbohydrates in the same material.

Materials and Methods

The genes sugary-1, brittle-1, and brittle-2 are recessive. At ma-

[1] Manuscript received October 2, 1953. California Institute of Technology and University of California Citrus Experiment Station. This work was partially supported by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, United States Navy (Contract N6-onr-244, Task Order 5, Project NR-164-340).

[2] Present address: U. S. Department of Agriculture, Federal Experiment Station, Mayaguez, Puerto Rico.

turity sugary kernels are shrunken, wrinkled, and translucent; sugary endosperms weigh about 75% as much as normal ones. Mature brittle-1 and brittle-2 kernels are quite similar in appearance. Both types have a brittle texture, are darker in color than normal, and are intermediate in wrinkling and translucency between sugary and normal; brittle-1 and brittle-2 endosperms respectively average 46 and 66% of normal in weight (12). A photograph of these mutant kernels has been published (12).

The details of the production of the genetic material have been described (4), but the essential points will be stated here. Lines homozygous for the recessive genes bt_1 and bt_2, respectively, were each crossed to a normal line of which more than seven-eighths of the germplasm was from the inbred CC5. The F_1 plants were selfed and resulting homozygous bt_1 and bt_2 kernels were planted in 1951. The plants thus obtained provided the ears used for the two developmental series. Ears homozygous for bt_1 were produced by mass sibbing of bt_1 plants. Ears with normal kernels were obtained by pollinating sister bt_1 plants by a normal line which was predominantly CC5. Kernels of bt_2 and its respective normal counterpart were obtained by corresponding pollinations. All kernel types thus contained a considerable proportion of CC5, but they were not highly isogenic, that is, were not identical with respect to genetic background. From 75 to 500 kernels, depending upon age, were collected at each sampling date. Embryos were removed and the endosperms were frozen, lyophilized, and stored below 0°C. Niacin was assayed as described by the Association of Vitamin Chemists (1). Each niacin value is the mean of four independent determinations. Tests for the significance of the difference between means at each sampling date were carried out by analysis of variance using the F test. For two samples, inadequate records of kernel weights were adjusted as described in the related paper (4).

The mature endosperm types described in Table I were derived from a cross of the bt_1 line with su_1. Selfing of the F_1 plants produced ears bearing normal, su_1, bt_1, and su_1 bt_1 kernels. Since bt_1 and su_1 bt_1 kernels are difficult to separate when they occur together, endosperms for the assays were obtained from the next generation, as follows. Kernels of normal and of su_1 phenotypes were grown and the plants were selfed. Among the ears obtained from the normals one was selected which was segregating for normal and su_1 kernels, and another which was segregating for normal and bt_1. From the su_1 plants an ear segregating for su_1 and su_1 bt_1 was taken. All kernel types, derived in this way, could be accurately separated. Niacin assays

were performed in quadruplicate, as with the developing kernel samples.

A similar study of the interaction of bt_2 with su_1 was desirable, but since these two genes appear to be closely linked (12), extraction of the double recessive was not attempted.

Results and Discussion

Developmental Series. Figure 1 shows the behavior of niacin in bt_1 endosperms and related normal (nonbrittle) ones during development. At 14 days the difference in niacin between bt_1 and normal was significant at the 5% level, but was very small. At 21 days there was no significant difference. From 25 days on, the differences were all significant at the 1% level.

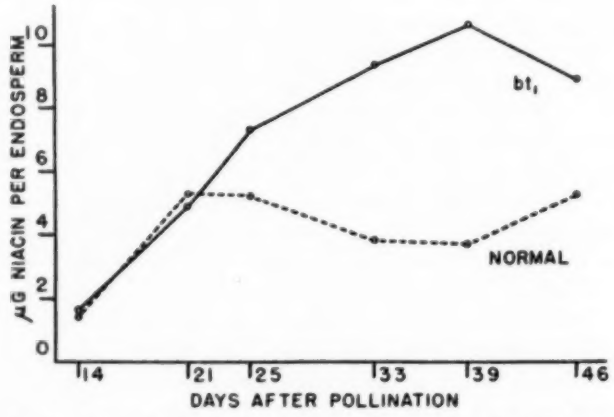


Fig. 1. The niacin content of developing endosperms of brittle-1 *(bt_1)* and a related normal genotype.

Figure 2 shows the behavior of niacin in bt_2 endosperms and in the related normal. As with bt_1, niacin was higher in bt_2 than in normal during all the later developmental period. The differences were significant at the 5% level at 14 days and at the 1% level from 21 days on. The general behavior of the two brittle genotypes was notably similar. In each the niacin content rose rapidly between 14 and 39 days and then decreased by 46 days. The two normal types were also fairly similar to one another, although, as indicated, their germplasms were not isogenic. In both, niacin reached a maximum at about mid-development and then decreased. In an earlier study (10) niacin behavior during kernel development was determined in two other normal lines

of maize, R4 and KYS. The niacin patterns in those lines were also similar to those found here. In the present normal line of the bt_1 series, niacin was higher at 46 days than at 39 days. This has not been found in the other lines and may represent fluctuation in an individual sample.

The dry weights (not shown) of both the bt_1 and bt_2 endosperms were considerably lower than those of corresponding normals from about 25 days on. Therefore the differences in niacin content on a weight basis were larger than on the per-endosperm basis. This has already been shown to be the case for mature kernels (11).

The behavior of niacin in bt_1 and bt_2 follows a pattern rather similar to that already reported for sugary (su_1) endosperms (10). In all three mutant types niacin becomes higher than in normals at about mid-development and remains so thereafter. Our data suggest that when genetic background is taken into account, bt_1 and bt_2 contain somewhat more niacin than does su_1, on a per-endosperm basis. Hunt *et al.* (5) reported, on a dry-weight basis, the niacin content of developing grain of four su_1 corn varieties. The present bt_1 and bt_2 data calculated on this basis showed higher niacin than that of the sugary varieties of these authors, throughout the sampling periods.

The Interaction of bt_1 with su_1. It was of interest to determine the effect of the double recessive genotype su_1 bt_1 upon niacin content of the endosperm. In order to obtain accurate separation of kernel types

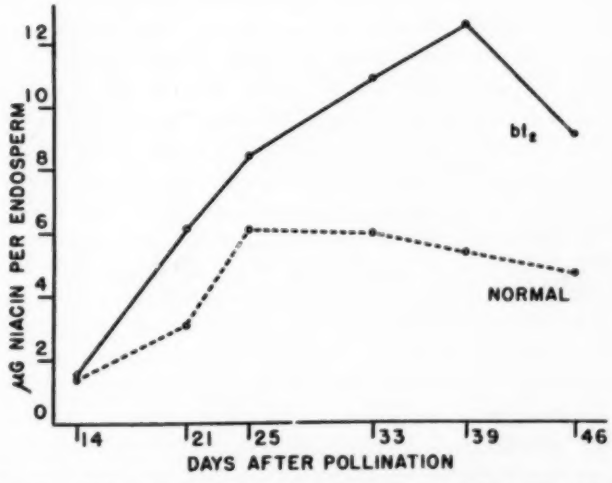


Fig. 2. The niacin content of developing endosperms of brittle-2 *(bt_2)* and a related normal genotype.

and a reasonably comparable genetic background, kernels from three related ears (see Materials and Methods) were assayed. Table I shows the air-dry weights of the endosperm types, and the niacin content on both a per-endosperm and a per-gram basis. On ear 1 niacin was higher in su_1 than in normal on both the per-endosperm and the per-gram bases and the differences were significant at the 5% and 1% levels, respectively. On ear 2 niacin was higher in bt_1 than in normal and the differences on both bases were significant at 1%. Ear 3 afforded a direct comparison between su_1 and su_1 bt_1. On the per-endosperm basis niacin was not significantly different in the two types. Likewise, niacin was very little different in su_1 bt_1 than in bt_1 from ear 2. On the per-gram basis, however, the niacin content of su_1 bt_1 was 155.5 μg., which is much higher than that of any of the other types. To the authors' knowledge it is the highest niacin concentration yet reported for maize kernels. It may be noted that increased niacin was accompanied by decreased endosperm weight in the genotypes in Table I. The su_1 bt_1 endosperms were lightest of all, averaging only 45 mg. per endosperm. The higher niacin in sugary kernels from ear 3 compared to sugary kernels from ear 1 was unexpected. It may be that a heterozygous brittle plant, such as the one on which ear 3 was borne, provides a more favorable maternal environment for niacin accumulation by sugary kernels than a normal or sugary plant. Alternatively the higher niacin in sugary kernels on ear 3 might be due to an interaction of the brittle genes carried by two-thirds of the nonbrittle kernels. In either case niacin should be even further increased in sugary kernels from a

TABLE I

NIACIN CONTENT OF MATURE MAIZE ENDOSPERMS OF SUGARY-1, BRITTLE-1, AND RELATED GENOTYPES

Endosperm Genotype	Ear No.[a]	Air-Dry Weight per Endosperm	Niacin per Endosperm[b]	Niacin per g. Tissue[b]
		mg.	*μg.*	*μg.*
Normal	1	195	3.8	19.5
Sugary-1 (su_1)	1	162	4.8*	29.6**
Normal	2	191	3.1	16.2
Brittle-1 (bt_1)	2	85	7.6**	89.4**
Sugary-1 (su_1)	3	100	7.1	71.0
Sugary-1 Brittle-1 (su_1 bt_1)	3	45	7.0	155.5**

[a] Each ear was segregating only the two genotypes indicated.

[b] Asterisks indicate that differences from the accompanying genotype on the same ear are significant at 5% (*) or 1% (**).

homozygous brittle-1 ear segregating sugary. Unfortunately this type of ear was not obtained, because no seed of the appropriate genotype germinated.

Several previous studies of mutant kernel types (2,3,6,7,8,10,11) have demonstrated a correlation between high niacin content and various differences in stored carbohydrates. This correlation holds true for the present bt_1 and bt_2 material, as can be seen by comparing the present niacin data with the related data (4) on carbohydrates. High niacin in the two developmental brittle series was accompanied by less starch accumulation and a higher accumulation of sugars than in normals. Among the mature endosperm types of Table I, su_1 bt_1 contained less starch than any other.

As cited earlier (9), the higher niacin in mature su_1 endosperms has been shown to be largely localized in the aleurone layer, in cells which are larger than those in normal (starchy-1) aleurone. It may be that the same condition occurs in bt_1 and bt_2, and in other mutant kernel types.

In view of the well-known niacin deficiency of maize products, it is of interest to find a genetic combination (homozygous sugary-1 brittle-1) in which the niacin content is eight to ten times as high as the average field corn, even though the likelihood of practical advantage being taken of this information is small. However, it is possible that an endosperm mutant such as brittle-1 or brittle-2 might possess characteristics that would make it suitable for "roasting ears" or for special purposes as yet undetermined.

Literature Cited

1. Association of Vitamin Chemists. Methods of vitamin assay. Interscience: New York (1947).
2. Burkholder, P. R., McVeigh, Ilda, and Moyer, Dorothy. Niacin in maize. Yale J. Biol. and Med. **16:** 659–663 (1944).
3. Cameron, J. W., and Teas, H. J. The relation between nicotinic acid and carbohydrates in a series of maize endosperm genotypes. Proc. Natl. Acad. Sci. [U. S.] **34:** 390–398 (1948).
4. Cameron, J. W., and Teas, H. J. Carbohydrate relationships in developing and mature endosperms of brittle and related maize genotypes. Am. J. Bot. **41:** 50–55 (1954).
5. Hunt, C. H., Rodriguez, Lorraine D., and Bethke, R. M. The effect of maturity on the niacin and pantothenic acid content of the stalks and leaves, tassels, and grain of four sweet corn varieties. Cereal Chem. **27:** 157–161 (1950).
6. Leng, E. R., Curtis, J. J., and Shekleton, M. C. Niacin content of waxy, sugary, and dent F_2 segregating kernels in corn. Science **111:** 665–666 (1950).
7. Mather, K., and Barton-Wright, E. C. Nicotinic acid in sugary and starchy maize. Nature **157:** 109–110 (1946).
8. Richey, F. D., and Dawson, R. F. Experiments on the inheritance of niacin in corn (maize). Plant Physiol. **26:** 475–493 (1951).
9. Teas, H. J. A morphological basis for higher niacin in sugary maize. Proc. Natl. Acad. Sci. [U. S.] **38:** 817–822 (1952).

10. TEAS, H. J., CAMERON, J. W., and NEWTON, ANNA C. Tryptophan, niacin, indoleacetic acid, and carbohydrates in developing sugary and starchy maize kernels. Agron. J. 44: 434–438 (1952).
11. TEAS, H. J., and NEWTON, ANNA C. Tryptophan, niacin, and indoleacetic acid in several endosperm mutants and standard lines of maize. Plant Physiol. 26: 494–501 (1951).
12. TEAS, H. J., and TEAS, ANNA N. Heritable characters in maize: Description and linkage of brittle endosperm-2. J. Hered. 44: 156–158 (1953).

KINETIC STUDIES OF THE CATALASE SYSTEM OF WHEAT[1]

G. N. IRVINE, W. BUSHUK, AND J. A. ANDERSON

ABSTRACT

The catalase system of wheat endosperm was studied manometrically using water extracts as enzyme solution and hydrogen peroxide as substrate. Peroxide decomposition follows first-order kinetics over a wide range of enzyme and substrate concentrations. The first-order specific rate constant is directly proportional to enzyme concentration and independent of substrate. Effects of pH, temperature, cyanide, and high peroxide concentrations were determined. The system does not have a true Michaelis constant. Energy of activation between 10° and 25°C. is 2,800 calories. Crystalline catalase is readily inactivated by peroxide concentrations which have no effect on crude wheat catalase.

The widespread occurrence of catalase in nature has stimulated a vast amount of research on the various aspects of the reaction catalyzed by this particular enzyme. Although the mechanism of its action has been fairly well worked out, its biological role is still not too clearly understood. Catalase was first obtained in crystalline form in 1937 (8) and since then has been of great theoretical interest because complex formation with hydrogen peroxide, its specific substrate, has actually been demonstrated and studied by Chance (3). Using a recording microspectrophotometric technic, he has been able to investigate the kinetics of formation of the activated complex and its subsequent reaction with a second peroxide molecule to give products and free enzyme. It has generally been accepted that the function of catalase is to protect the living cell from the harmful effects of hydrogen peroxide. Recently, however, some doubt was cast on this view when Keilin and Hartree (6) and others (2, 9, 10) demonstrated that catalase, in the presence of small amounts of hydrogen peroxide, can oxidize certain compounds such as alcohols and phenols. It has been suggested that this enzyme is actually a special type of peroxidase (10).

[1] Manuscript received December 23, 1953. Paper No. 131 of the Grain Research Laboratory and No. 312 of the Associate Committee on Grain Research (Canada). Based in part on a thesis submitted by W. Bushuk (recipient of a National Research Council Bursary) to the University of Manitoba in partial fulfillment of requirements for the degree of M.Sc., 1953.

The work reported in this paper was undertaken to compare the kinetics of crude wheat catalase extracts with the kinetics which have been demonstrated for crystalline animal catalase. Our particular interest in the reaction catalyzed by this enzyme arises from the possibility that it may be involved in the oxidation reactions which are postulated to occur during dough development. In an attempt to reproduce natural conditions as closely as possible, the active enzyme extract as obtained from wheat endosperm was not altered by attempts to concentrate or purify it.

Materials and Methods

Preparation of the Enzyme. Enzyme extracts were prepared from semolina milled from a single pure variety of durum wheat, Pelissier. Durum semolina was selected because it was readily available and was being used at the time in other enzyme studies at this Laboratory. Middlings or flour milled from bread wheats are of comparable catalase activity and could have been used instead. A discussion of the relative activities of various types of wheat will be presented in a later paper.

Active enzyme solutions were obtained by grinding 10.0 g. of semolina and 5.0 g. of acid-purified sand with 20 ml. of distilled water for 5 minutes in a mechanical mortar grinder. The mass was then transferred into a 50-ml. round-bottom centrifuge tube and spun at top speed for 10 minutes in a clinical centrifuge. The supernatant liquid constitutes the active extract. Enzyme concentrations are expressed in terms of milliliters of this extract. These preparations were found to be highly reproducible from day to day and accordingly were made up fresh daily.

Preparation of the Substrate. The hydrogen peroxide solution used as substrate in this study was prepared by diluting a small amount of superoxol (Merck) with distilled water. Approximately 0.045 *M* solutions were used in routine experiments. Hydrogen peroxide of this concentration shows only a slight decrease in concentration after several weeks when stored at 5°C. If 1.0 ml. of this preparation is used in a total fluid volume of 7.0 ml., the initial peroxide concentration is about 0.006 *M*.

Preparation of Solution of Crystalline Catalase. Crystalline catalase was obtained from General Biochemicals Inc. An active solution was prepared by dissolving 2.5 mg. of the crystalline enzyme in 240 ml. of distilled water and 10 ml. of 0.067 *M* phosphate buffer (pH 7.3). The activity of this preparation was too high to be measured accurately in

the Warburg apparatus; accordingly it was diluted to approximately one-tenth concentration before use.

Method. Measurements of oxygen liberated were made in a Warburg apparatus with standard 50-ml. reaction vessels. Enzyme solution was usually placed in the side arm and the substrate and buffer solutions in the main compartment. The total fluid volume was always adjusted to 7.0 ml. with suitable amounts of 0.067 *M* phosphate buffer. In the inhibition experiments, inhibitor and enzyme were placed in the main compartment and substrate was pipetted in the side arm.

The flasks were equilibrated by shaking in the bath for 10 minutes, and the reaction initiated by tipping the side arm contents into the main compartment. Shaking rate was 130 oscillations per minute at an amplitude of 4.0 cm. Except where indicated, the experimental temperature was 25.0°C.

Results

Typical reaction curves representing the amount of oxygen evolved as a function of reaction time for a range of enzyme concentrations are shown in Fig. 1. These data are replotted in Fig. 2 to show the

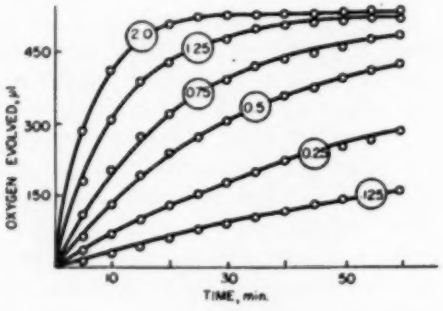


Fig. 1. Reaction curves for a range of enzyme concentrations.

relation of the logarithm of residual substrate concentration against time. The linearity of the latter plots indicates that under these particular experimental conditions the decomposition of hydrogen peroxide by wheat catalase proceeds according to first order kinetics.

Specific Rate Constant. Actual experimental data for a single run using 1.0 ml. of enzyme solution with approximately 0.006 *M* peroxide

$$k = \frac{2.303}{t} \log \frac{a}{a - x}$$

are shown in Table I. The first order rate constant was calculated using the equation

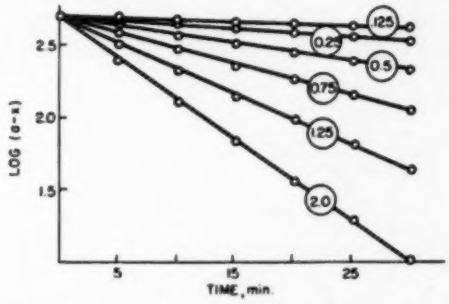


Fig. 2. First order plots for reactions represented by Fig. 1.

in which t is the time in seconds, a is the initial peroxide concentration, and x is the amount decomposed after time t. Accordingly, a — x represents the remaining substrate concentration at time t. For convenience in calculations, a and x may be expressed in terms of microliters of oxygen. Under these conditions the rate constant remains essentially the same beyond 80% decomposition of the peroxide. That is, up to that point, there is no evidence of inhibition by substrate or reaction products. The rate constant is directly proportional to enzyme concentration; accordingly it can be used as an index of the enzymic activity. Further evidence for this will be discussed in a later section of this paper. For enzyme reactions which follow first order kinetics, the observed rate constant is not the specific rate constant but related to it by

$$k' = k\,E$$

where k′ is the observed rate constant, k is the specific rate constant, and E is the enzyme concentration.

TABLE I

Enzymic Decomposition of Hydrogen Peroxide at 25°C., pH 7.3

t, seconds	a — x, μl. of O_2	k × 10^3, $sec.^{-1}$
0	466	...
300	343	1.02
600	244	1.08
900	180	1.06
1200	130	1.06
1500	96	1.05
1800	71	1.05

Hydrogen-Ion Concentration. Figure 3 shows the variation in the first order rate constant with pH. Optimum rate occurs at about pH 7.3 and consequently this was taken as the working pH for all other experiments. A similar optimum has been found by other investigators for crystalline catalase from animal sources.

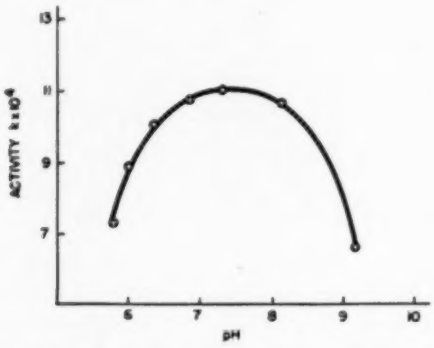


Fig. 3. Variation of rate constant (enzymic activity) with pH.

Temperature. Between 10° and 25°C. the reaction velocity varies in accordance with the Arrhenius equation. Over this temperature range the activation energy for the over-all reaction is 2,800 calories. Although this value is similar to recorded values for other catalase preparations, its accuracy may be limited by the small temperature interval on which the calculation is based. A decrease in the rate is observed above 25°C. which is probably not due to direct thermal destruction of the enzyme, as is the case with some enzymes, but to increased rate of formation of an inactive enzyme-substrate complex. Incubation of the active enzyme solution for 1 hour at 30°C. was found to have no significant effect on the activity. For catalase systems which do not follow first order kinetics, the optimum temperatures observed are usually considerably lower than that observed in this investigation.

Substrate Concentration. For enzyme concentrations in the range used in this investigation, the reaction is first order up to peroxide concentrations of about 0.01 *M*. Under these conditions the initial velocity of the reaction is directly proportional to the substrate concentration. Moreover, the first order rate constant is independent of the substrate concentration; this is shown by the log plot in Fig. 4, in which curves for the different substrate concentrations are essentially parallel.

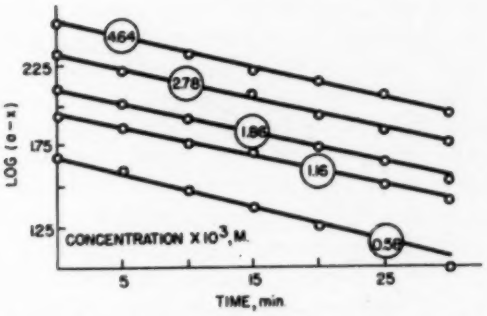


Fig. 4. First order rate curves at various substrate concentrations.

Figure 5 shows that a plot of the logarithm of the instantaneous rate against the logarithm of the residual substrate concentration gives a straight line of slope equal to one. That is, the order with respect to peroxide concentration (the true order of the reaction) is the same as the order with respect to time. This suggests that the reaction products have neither an activating nor an inhibiting effect on the enzyme.

At substrate concentrations in excess of about 0.01 *M*, inhibition of the enzyme by the substrate becomes apparent. A plot of initial velocity against substrate concentration, shown in Fig. 6, is no longer linear;

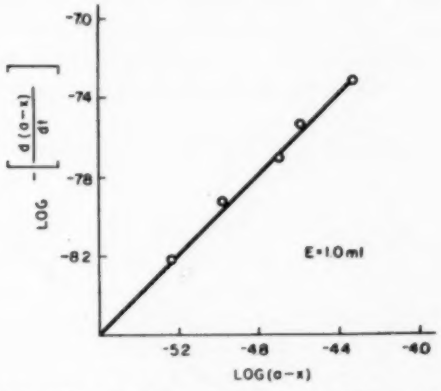


Fig. 5. Relation of logarithm of instantaneous rate to logarithm of substrate concentration.

the velocity falls off at the high substrate concentrations. In this region the first order rate constant for a given reaction is no longer a true constant but falls off with increasing reaction time. This suggests that the active enzyme concentration is decreasing. If the decrease in the

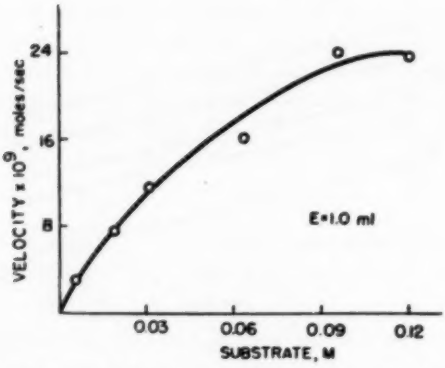


Fig. 6. Initial velocity as a function of substrate concentration in the region of high substrate concentrations.

initial velocity were due to saturation of the enzyme, then the reaction should be zero order with respect to time at the high substrate levels. Our results indicate, on the contrary, that the apparent order of the reaction becomes higher, i.e., the enzyme concentration as well as the substrate concentration is decreasing with time.

From a plot of initial velocity vs. substrate concentration in the region where the rate falls off (at high substrate concentration), a pseudo-Michaelis constant may be evaluated; however, it is found that this value increases with enzyme concentration. A number of investigators have quoted a Michaelis constant for the catalase system (4, 5, 7), but current opinion is that no true Michaelis constant exists for this system. The results obtained in this investigation support this latter view.

Enzyme Concentration. The reaction remains kinetically first order

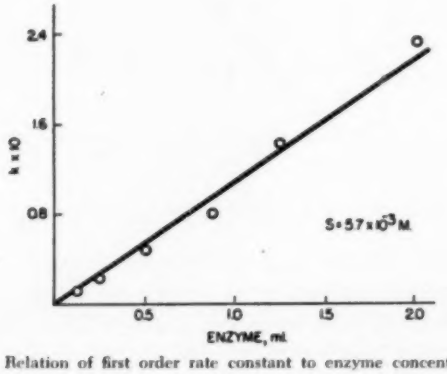


Fig. 7. Relation of first order rate constant to enzyme concentration.

over a wide range of enzyme concentrations, and the first order rate constant, which is directly proportional to the slopes of the curves in Fig. 2, varies linearly with enzyme concentration. Typical experimental results for a 16-fold variation in enzyme concentration are shown in Fig. 7. The direct proportionality between enzyme concentration and the rate constant provides a convenient means of expressing catalase activity of wheat and wheat products directly in terms of the constant. It has been shown earlier that the rate constant is independent of substrate concentration. Additionally, the rate constant may be used as a measure of activity in other studies.

Inhibition by Cyanide. The effect of low concentrations of cyanide ion is to decrease the active enzyme concentration. Since the reaction does not fit Michaelis-Menton kinetics, the orthodox methods for determining the characteristics of the inhibition are not applicable. The results of varying the substrate concentration at a fixed enzyme level in the presence and absence of cyanide ion are given in Table II.

TABLE II

INHIBITION OF CATALASE ACTIVITY BY CYANIDE AT VARIOUS SUBSTRATE CONCENTRATIONS

Initial Substrate (*M*)	Initial Velocity $\times 10^5$ (moles/sec.)		Inhibition
	No Cyanide	$2.50 \times 10^{-4}M$ Cyanide	
			%
0.0100	5.13	4.62	9.94
0.0090	4.63	4.11	11.23
0.0060	3.12	2.40	23.08
0.0050	2.51	1.77	29.48
0.0045	2.25	1.49	33.78
0.0035	1.79	1.01	43.58
0.0025	1.40	0.62	55.71

The percentage inhibition decreases with increasing substrate concentration and accordingly the inhibition appears to be competitive; that is, the substrate and inhibitor compete for the same active site. The percentage inhibition is constant when only the cyanide concentration is varied and also when the enzyme concentration alone is varied. The value of K_i was determined from a relation of the type

$$K_i = \frac{ES}{EI} \cdot I$$

in which ES is proportional to the initial velocity in the presence of cyanide and EI is proportional to the difference between the initial

velocity in the absence and in the presence of cyanide; I is the cyanide concentration under the condition that $I >> E_o$. At low substrate concentrations the value of K_1 is approximately 1.0×10^{-4} but at higher substrate levels (those of Table II) it increases approximately exponentially with substrate concentration.

Further work on cyanide inhibition is proceeding and will be published in a further paper.

Comparison with Crystalline Catalase. A plot of the logarithm of substrate concentration against time for a typical reaction using crystalline catalase as the active enzyme is represented by curve 1, Fig. 8.

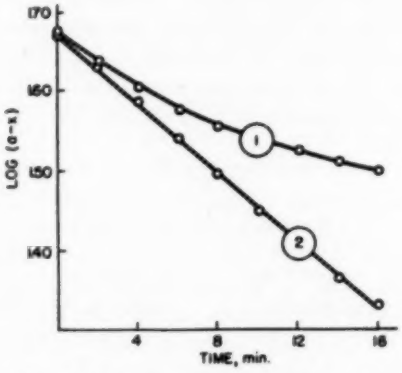


Fig. 8. Rate curves for crystalline catalase.

The reaction is not of the first order type that has invariably been observed with crude extracts of wheat endosperm. Our results suggest that decomposition of hydrogen peroxide by crystalline catalase proceeds according to apparently second order kinetics with respect to time; i.e., the enzyme is being consumed during the reaction. The decrease in the slope of curve 1, Fig. 8, may be taken as a direct measure of the decrease in the active enzyme concentration. It is apparent that the enzyme is being inactivated, since under certain conditions the rate becomes zero long before all the substrate is used up.

Curve 2, Fig. 8, shows the type of curve that is obtained when we add to the crystalline enzyme solution a small amount of enzymatically inactive filtrate from boiled wheat extracts. Inactivation of the enzyme is prevented, and the reaction under these conditions is first order. This observation is especially pertinent to any discussion of the differences between crude and purified catalase preparations.

Discussion

The results reported in this paper stress the importance of using crude enzyme extracts containing the water-soluble components of dough rather than purified enzyme preparations, if the object is to learn something about the behavior of the enzyme in a dough medium. It is evident that the water extracts of wheat endosperm, in addition to catalase, contain some substance which prevents the formation of an inactive complex at relatively low substrate concentrations. This thermo-stable component is probably an essential part of the natural enzyme system but is apparently removed in the process of preparing the crystalline enzyme.

Effects of temperature, high substrate concentrations, and cyanide can be explained by postulating: that two different types of active centers exist on the enzyme; that attachment of a molecule of hydrogen peroxide at site I forms an active complex, whereas an inactive complex is formed if the peroxide combines at site II; and that the function of the protective component in the wheat catalase system is to lower the activation energy requirement for attachment at site I over that for site II.

The mechanism of the postulated protective action is not readily evident from our results, although what possibly happens is that site II is masked by this component which is displaced at high peroxide concentrations. The formation of the inactive enzyme-substrate complex is apparent only at relatively high substrate concentrations. Higher temperatures either favor the formation of the inactive complex at site II or the dissociation of site I complex. Either effect would decrease the rate above a certain temperature. Formation of the inactive enzyme-cyanide complex could occur at site I, since the inhibition is competitive.

In general, the manometric results agree with the spectrophotometric results obtained by Beers and Sizer (1) for animal catalase. The over-all results are readily explainable on the basis of the consecutive reaction mechanism scheme postulated by Chance (3). The first order rate equations may be theoretically derived from the reaction mechanism by either the equilibrium or the steady-state treatments.

Literature Cited

1. Beers, R. F., Jr., and Sizer, I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195**: 133–140 (1952).
2. Boeri, E., and Bonnichsen, R. K. Oxidation of thiol groups by catalase. Acta Chem. Scand. **6**: 968–969 (1952).

3. CHANCE, B. The iron-containing enzymes. C. The enzyme-substrate compounds and mechanism of action of the hydroperoxidases. *In* The enzymes, ed. by J. B. Sumner, Vol. II, Part I. Academic Press: New York (1951).
4. EULER, H. VON, and JOSEPHSON, K. Catalase II. Ann. **455:** 1–16 (1927). (Chem. Abstr. **21:** 3377 (1927).)
5. HENNICHS, S. Liver catalase. Biochem. Z. **145:** 286–305 (1924). (Chem. Abstr. **19:** 84 (1925).)
6. KEILIN, D., and HARTREE, E. F. Properties of catalase. Catalysis of coupled oxidation of alcohols. Biochem. J. **39:** 293–301 (1945).
7. SHIRAKAWA, M. Crystalline catalase. IX. Consideration on the combination with the substrate. J. Agr. Chem. Soc. Japan **25:** 166–171 (1951). (Chem. Abstr. **46:** 11296 (1952).)
8. SUMNER, J. B., and DOUNCE, A. L. Crystalline catalase. Science **85:** 366–367 (1937).
9. TAUBER, H. Crystalline catalase, a peroxidase. Proc. Soc. Expt. Biol. and Med. **81:** 237–239 (1952).
10. THEORELL, H. The iron-containing enzymes. B. Catalases and peroxidases. "Hydroperoxidases." *In* The enzymes, ed. by J. B. Sumner, Vol. II, Part I. Academic Press: New York (1951).

NOTE ON THE CATALASE ACTIVITY OF NORTH AMERICAN WHEATS[1]

G. N. Irvine, W. Bushuk, and J. A. Anderson

ABSTRACT

Catalase activities of flour or semolina from sixteen wheat varieties belonging to five classes were determined manometrically; activities were expressed in terms of the first order rate constant. Flour activities varied from 3.09×10^{-8} sec.$^{-1}$ to 0.32×10^{-8} sec.$^{-1}$ and semolina activities from 1.04×10^{-8} sec.$^{-1}$ to 0.44×10^{-8} sec.$^{-1}$. Hard red spring wheat flours were generally distinctly higher than those from other classes. Variation of catalase activity with variety and environment within one class (durum) was investigated with a series of seven varieties grown at seven stations. Variation between varieties was as high as threefold, between stations as high as fourfold. Catalase activity of ground whole wheat is approximately six times that of semolina.

One of the first studies of the catalase activities of North American wheat flours was reported by Bailey (1) in 1917. He observed that catalase activity increased with flour ash and suggested that catalase measurements might be employed in place of the ash determination as an index of flour grade. Some years later Blish and Bode (2) determined the catalase activity of a series of 33 commercial flours obtained from different mills in the United States and Canada. Although the wheats used in the flours were not known, their results suggested that environment and type of wheat used far outweighed ash variation in determining catalase activity. From a restricted series of five varieties grown under identical conditions, they observed marked differences between spring wheats and winter wheats but little difference among different varieties in each of these classes.

The investigation reported in this paper originated from the kinetic study of the catalase system of wheat that has been described previously (3). A large number of durum varieties were tested as a likely source of the enzyme for the kinetic study and a wide variation in activity was observed. This prompted a more extensive survey covering pure varieties belonging to several classes of wheat and a study of the variation within one class (durum) of activity with variety and environment. The results are presented here.

Materials and Methods

All samples used in this investigation represent pure varieties

[1] Manuscript received January 18, 1954. Paper No. 133 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and No. 313 of the Associate Committee on Grain Research (Canada). Based in part on a thesis submitted by W. Bushuk (recipient of a National Research Council Bursary) to the University of Manitoba in partial fulfillment of the requirements for the degree of M.Sc., 1953.

grown at various stations in Western Canada and the United States. Flours and semolinas were freshly milled for this investigation. Wheats other than durum were milled in the laboratory to yield a straight flour of 70% extraction; durum wheats were milled into semolina of 50% extraction. Whole wheats were ground as finely as possible on the Labconco mill.

Enzyme extracts were prepared from 10.0 g. of flour, semolina, or ground whole wheat by the procedure outlined in a previous paper (3). The activity of 1.0 ml. of undiluted extract acting on 1.0 ml. 0.045 *M* hydrogen peroxide was determined in a Warburg apparatus. Total fluid volume was adjusted to 7.0 ml. with 0.067 *M* phosphate buffer of pH 7.3. The temperature of the bath was 25.0°C.

Rates of hydrogen peroxide decomposition were obtained from manometric measurements of oxygen evolved during the reaction and the enzymic activity was expressed in terms of the first order rate constant calculated from the data. The traditional *Kat.f.* value is used largely to express purity of catalase preparations and since the extracts used in this work were of very low activity compared to purified preparations it would have little significance. One milliliter of wheat extract as used in this work represents 0.5 g. of material and accordingly the activity values quoted in Tables I and II are related to *Kat.f.* by

$$Kat.f. = \frac{\text{activity value} \times 10^{-3}\,\text{sec.}^{-1}}{2.303\,(0.5)}$$

As listed in the tables for ease of comparsion, the activity values must be multiplied by 10^{-3} sec.$^{-1}$ to obtain the measured first order rate constant. The units of this rate constant are reciprocal seconds. (The units of *Kat.f.* are reciprocal seconds per gram.)

Table I lists the catalase activities of 16 wheat varieties representing five different classes. The reproducibility varies with the activity, variation being of the order of ± 0.01 for the low values and ± 0.05 for the high values. These results confirm the suggestion of Blish and Bode (2) that variation is considerably less between wheats of the same class than between classes, and that winter wheats have markedly lower catalase activity than spring wheats. The lower activity of the durums as compared to the spring wheats might well be accounted for by the lower extraction used in milling semolina.

A second investigation was then made to investigate more fully the variation of catalase activity between wheats of the same class, and also the effect of environment. A series of durum wheats, repre-

senting seven varieties grown at seven experimental stations in Western Canada, was used in this study. Catalase activities of both wheat and semolina were determined, and the varietal and station means for these are given in Table II.

TABLE I

CATALASE ACTIVITY OF FLOUR OR SEMOLINA FROM VARIOUS NORTH AMERICAN WHEAT VARIETIES

Class of Wheat	Variety	Catalase Activity ($\times 10^3$)sec.$^{-1}$
Hard red spring	Lee	3.09
	Marquis	2.92
	Thatcher	2.56
	Redman	1.91
Hard red winter	Comanche	0.42
	Pawnee	0.32
Soft red winter	Thorne	0.74
	Vigo	0.42
White wheat	Golden	2.30
	Baart	1.33
	Idaed	0.88
	Elmar	0.85
Amber durum	Golden Ball	1.04
	Nugget	0.72
	Carleton	0.66
	Mindum	0.44

The reason for the great sensitivity of catalase activity of flours to degree of extraction and ash content for a given sample of wheat is apparent from a comparison of the wheat and semolina catalase values. Wheat is, on the average, about six times as active as semolina while the difference in ash is about 2½-fold.

Variation of catalase activity is somewhat greater between stations

TABLE II

MEAN CATALASE ACTIVITY OF DURUM WHEATS AND SEMOLINAS

Variety	Wheat	Semolina	Station	Wheat	Semolina
	($\times 10^3$)sec.$^{-1}$			($\times 10^3$)sec.$^{-1}$	
Golden Ball	6.17	1.06	Saskatoon	6.04	0.84
D. T. 125	5.03	0.86	Lethbridge	5.37	0.90
D. T. 122	4.72	0.80	Morden	4.88	0.64
Nugget	4.30	0.73	Melita	4.45	0.65
Carleton	4.43	0.68	Swift Current	4.30	1.11
D. T. 208	3.55	0.57	Brandon	4.06	0.62
Mindum	3.55	0.46	Indian Head	2.67	0.39

than between varieties; statistical analysis of these data indicates that between stations and between varieties, for both wheat and semolina, the variations are all highly significant. It is evident from Table II that the correlation between wheat and semolina activity is high between varieties but low between stations; the over-all correlation coefficient is only 0.66**. Between variety means the correlation is sufficiently high to predict semolina activity from measurements of wheat activity but between station means this cannot be done. The over-all correlation is likewise too low to permit estimation of semolina activity from wheat activity values for single samples.

These results may be compared with a similar study of lipoxidase activity for this same series of samples that was recently reported (4). Variation of lipoxidase activity with environment was very small and barely significant statistically, while varietal variation was very high. It may be concluded that different enzyme systems of wheat may vary quite independently with variety and environment. Protein content was determined for this series and no relation between protein content and catalase activity was found.

Literature Cited

1. Bailey, C. H. The catalase activity of American wheat flours. J. Biol. Chem. **32**: 539–545 (1917).
2. Blish, M. J., and Bode, C. E. Catalase activity in wheat flour. Cereal Chem. **12**: 133–142 (1935).
3. Irvine, G. N., Bushuk, W., and Anderson, J. A. Kinetic studies of the catalase system of wheat. Cereal Chem. (in press).
4. Irvine, G. N., and Anderson, J. A. Variation in principal quality factors of durum wheats with a quality prediction test for wheat or semolina. Cereal Chem. **30**: 334–342 (1953).

DETECTION OF INTERNAL INSECT INFESTATION IN GRAIN BY SOUND AMPLIFICATION[1]

R. E. ADAMS,[2] J. E. WOLFE,[2] MAX MILNER,[3] and J. A. SHELLENBERGER[3]

ABSTRACT

An electronic amplification technic to permit detection of sounds produced by insects infesting wheat internally has been devised. Noises produced by rice weevil larvae and pupae include sounds of low frequency apparently indicative of movement within the kernels, and also some of high frequency characteristic of larval feeding. Proposed applications of this method include evaluation of effectiveness of fumigants on insect stages developing within kernels, and the monitoring of infestation in bins of stored grain.

Detection of hidden insect infestation in grain kernels as a means to segregate and eliminate, prior to milling, grain that is excessively contaminated by insects, is now widely practiced in flour mills in order to minimize the formation of insect fragments. Kernels which contain hidden infestation of this type and which cannot be removed by grain-cleaning operations are acknowledged to be the primary source of insect fragments in the milling process (6). A discussion of these problems and a review of means currently available to millers to achieve low-fragment counts in flour have been published (3, 7). A rigorous evaluation of several of these technics has recently been made (12).

Tests for the detection of hidden internal infestation in commercial wheat which have been proposed include presumptive tests such as staining (4, 5, 8), a liquid flotation separation of insect-emerged kernels (2), isolation of insect parts for counting and identification following so-called cracking and flotation methods (6), separation by blowing kernels from which insects have emerged (9), and direct visualization of hidden infestation by radiography (10, 11).

The present report, which is one of a continuing series from this station dealing with the development of inspection technics for internally infested grain (8, 9, 10, 11), suggests the use of electronic amplification of sounds produced by insects such as the granary weevil and rice weevil, which develop in grains internally. A preliminary report of this technic has been published (1).

The major parts of the apparatus developed for this work include an amplifier, microphone, power supply, and a soundproof box, to-

[1]Manuscript received May 27, 1953. Presented at the Annual A.A.C.C. Meeting, May, 1953. Contribution No. 243, Department of Flour and Feed Milling Industries, Kansas State College, Manhattan, Kansas.

[2] Department of Electrical Engineering.
[3] Department of Flour and Feed Milling Industries.

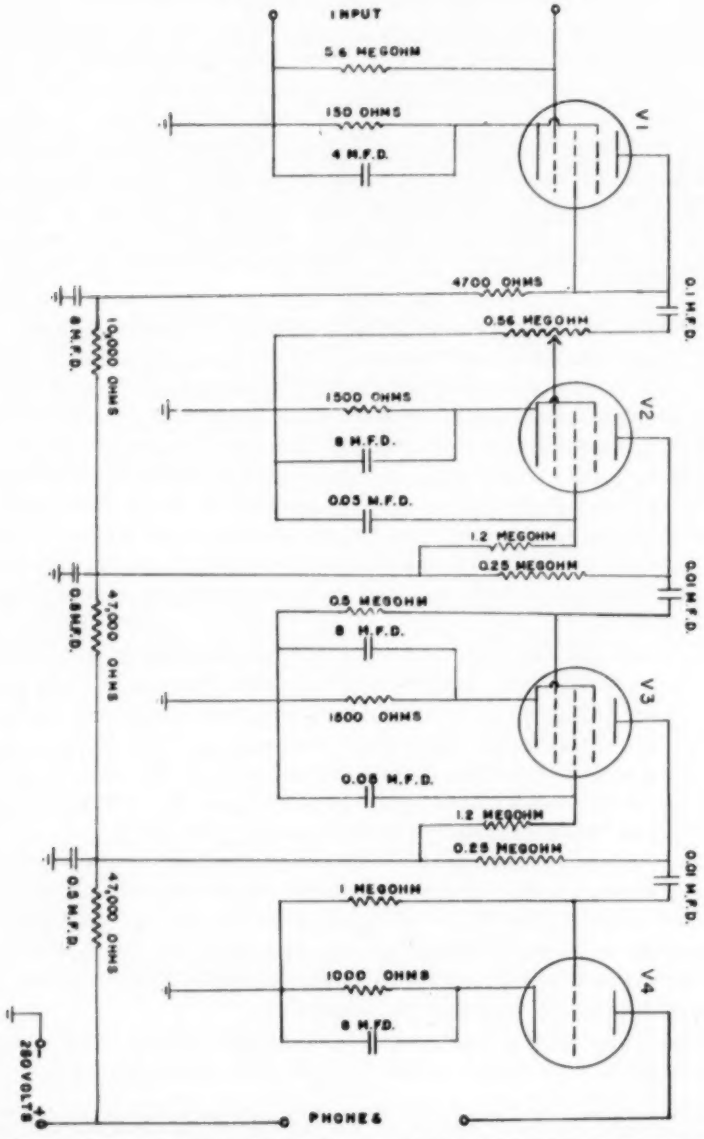


Fig. 1. Circuit of amplifier used for detection of hidden insect infestation in grain. Tube types used are as follows: V-1, 6AC7; V-2 and V-3, 6SJ7; V-4, 6J5.

gether with auxiliary equipment for analyzing the sound patterns, such as a Tektronix oscilloscope with a Polaroid-Land attachment for recording the sound patterns produced. A detailed description of the apparatus follows.

Amplifier

Preliminary investigations suggested that amplifiers of very high gain and low noise level would be required for these investigations, and a voltage gain of nearly 10,000,000 was projected. However, it soon became apparent that insect sounds were of sufficient magnitude that such great amplification was unnecessary. Accordingly, a circuit with less gain (about 77,000) but with very low noise-to-signal ratio was built. The circuit used is shown in Fig. 1. Tube V-1, shown in the diagram, is a 6AC7 triode, selected because of its low noise level. Tubes V-2 and V-3 are both type 6SJ7's which have high gain characteristics, the signal-to-noise ratio being determined by the type 6AC7 (V-1). Tube V-4 is a 6J5 used for impedance matching to the earphones; other circuit components such as resistors and condensers are standard types with 10% tolerance of stated values. The metal amplifier box is of standard construction and afforded adequate shielding for satisfactory performance. The soundproof concrete box contained copper shielding to prevent hum pickup from the power system.

The amplifier was constructed to use headphones but later a speaker was connected by adding an output transformer to the circuit.

Soundproof Box

The design of the soundproof box turned out to be an exacting problem since, with the amplification employed, vagrant sounds of very low intensity originating in the laboratory or the building interfered with the detection of sounds due to insects. A box constructed of several layers of fiber wallboard with air spaces between the layers proved to be inadequate. Finally a concrete box was constructed in the form of a cube with edges 2 ft. long and with walls and removable cover 2½ in. thick. Within this box was a copper container to provide electrical shielding, and within this metal container were placed two smaller boxes of fiber wallboard. The innermost boxes contained a Western Electric hearing-aid microphone attached to a small metal cup, within which the grain is placed.

Portable Sound Detection Apparatus

An outgrowth of the original apparatus used for laboratory investi-

gations described above was a compact portable device pictured in Fig. 2. This apparatus consisted of three components, namely an in-

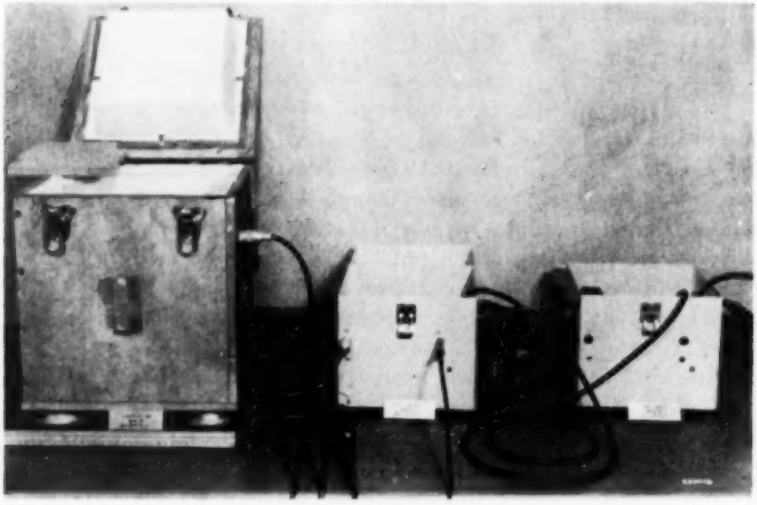


Fig. 2. Portable sound detection apparatus.

sulated box, a power supply, and an amplifier, which are plugged together with cables for use. The insulated box is a 12-in. cube made from cast ribbed aluminum plates ¾ in. in thickness for outer walls, and fluffed crepe paper insulation on the inner side walls, the bottom, and the hinged top. The innermost chamber is a 5½-in. cube containing a Western Electric microphone upon which is fastened a thin metal plate 4 in. square with turned-up edges. This permits aural examination of 50 g. of grain in a single layer. The whole assembly can be easily transported and operated in the luggage compartment of an automobile, and requires only plugging in to a standard 110-volt receptacle.

Description of Insect Sounds

Two distinct types of sound are associated with the larval and pupal stages, namely, a low-frequency scraping noise and a high-frequency tearing or rasping sound. From repeated observations it has been deduced that the low-frequency sounds are made by the movement of larva and pupa within the kernel, and the high-frequency sounds by the chewing of the endosperm of the grain by the larva. When several infested kernels are placed on the microphone, com-

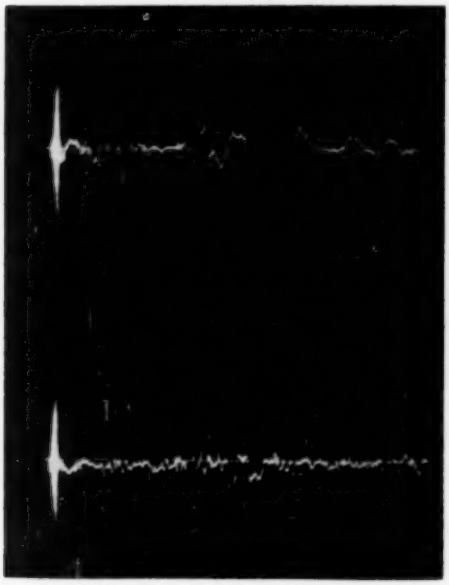


Fig. 3. Oscilloscope patterns of noises of internal insect infestation in wheat grains.

binations of these frequencies may appear, as shown in Fig. 3. Figure 3 was taken with the oscilloscope set at a sweep frequency of about 30 cps, and the insect sounds of both high and low frequencies are present. Thus in the upper trace the right and left ends show the low noise frequencies centering around 200 cps, characteristic of insect movement. Just to the right of the vertical scale in the center appears a high-frequency burst of sound in the range of 1,200–1,500 cps. The frequency range of sounds due to internal insects appears to range from 200 to 8,000 cps, although the lower limit has not been accurately determined. The noise level of the amplifier system produces negligible deflection with the gain used in recording these oscillograms. The voracious eating habits of the larval stage of rice weevil (*Sitophilus oryza* L.) has been clearly confirmed by this technic. It was also of interest to learn that, when the infested grains are disturbed in any way, the high-frequency sounds indicative of chewing usually cease and the low-frequency sounds, due apparently to movement, continue intermittently. After a short time the high-frequency sounds reappear. An experienced observer can estimate the approximate stage of development of the insect because the sounds are slightly different in the larval and pupal stages. This observation has suggested analysis of the recorded sound-wave patterns as a means for differentiating develop-

mental stages as well as physiological activities. Additional studies now in progress include evaluation of the method to determine numbers of infested kernels on the basis of cumulative recording of wave peaks, analysis of differences in sound characteristics of different species which infest grain internally, relationship of frequency of feeding and movement to stage of insect development, and determination of the influence of storage temperature and humidity on the nature, frequency, and periodicity of the sound patterns produced.

One practical application of this work is a means for the rapid evaluation of the effectiveness of fumigants whereby the normal delay of several weeks required for emergence of surviving insects, now necessary to determine fumigant efficiency, can be eliminated. Another application of the principle would be a means for monitoring grain within storage bins for infestation without sampling or removing the grain from the bins, in much the same manner as permanent thermocouple systems are now used for checking the heating of grain in storage. Work in progress suggests that selected frequency bands specific for the insect sounds can be detected, thus a means may be provided to minimize ordinary extraneous noises not caused by live infestation.

Literature Cited

1. Adams, R. E., Wolfe, J. F., Milner, M., and Shellenberger, J. A. Aural detection of grain infested internally with insects. Science **118:** 163–164 (1953).
2. Apt, A. C. A rapid method of examining wheat samples for infestation. Milling Production (May 1952).
3. Farrell, E. P., and Milner, M. Insect fragment problem in the milling industry. Kansas Agr. Expt. Sta. Circ. 291 (Nov. 1952).
4. Frankenfeld, J. C. Staining methods for detecting weevil infestation in grain. U. S. Dept. Agr., ET-No. 256 (1948).
5. Goossens, H. J. A method for staining insect egg plugs in wheat. Cereal Chem. **26:** 419–420 (1949).
6. Harris, K. L., Nicholson, J. F., Randolph, Lila K., and Trawick, J. L. An investigation of insect and rodent contamination of wheat and wheat flour. J. Assoc. Off. Agr. Chem. **35:** 115–158 (1952).
7. Milner, M. Recent developments in methods for detecting internally infested wheat. Milling Production (Jan. 1951).
8. Milner, M., Barney, D. L., and Shellenberger, J. A. Use of selective fluorescent stains to detect insect egg plugs on grain kernels. Science **112:** 791–792 (1950).
9. Milner, M., Farrell, E. P., and Katz, R. Use of a simple blowing device to facilitate inspection of wheat for internal infestation. J. Assoc. Off. Agr. Chem. **36:** 1065–1070 (1953).
10. Milner, M., Katz, R., Lee, M. R., and Pyle, W. B. Application of the Polaroid-Land process to radiographic inspection of wheat. Cereal Chem. **30:** 169–170 (1953).
11. Milner, M., Lee, M. R., and Katz, R. Application of X-ray technic to the detection of internal insect infestation of grain. J. Econ. Entomol. **43:** 933–935 (1950).
12. Reed, G. L., and Harris, K. L. An evaluation of five procedures for the determination of internal insect infestation of wheat. J. Assoc. Off. Agr. Chem. **36:** 138–159 (1953).

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **45:** VII–CCLV (1951).

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/16 to 1/8 inch high after reduction.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* **3**: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

ERRATUM

Cereal Chemistry, Vol. 31, No. 2

(March, 1954)

Page 127, ROBERTS *et al.* *For* line 7, paragraph 2, *please substitute:*

. . . blue values and total soluble starch become higher as the steaming . . .

The sentence will then read:

"Results indicate that the starch-iodine blue values and total soluble starch become higher as the steaming temperature is increased and that the steeping temperature and time have a lesser effect on these values."

STERWIN PRESENTS
a BETTER Chlorinator

Another Development of Sterwin's continuous research

A precision machine providing, for the first time, accurate and individual metering of chlorine to each agitator. Guesswork and proportioning are eliminated. Tested and proven by long-time, successful mill operation.

ACCURATE CONTROL
MINIMUM ADJUSTMENT
UNIFORM RESULTS
GREATER ECONOMIES
WIDE VERSATILITY
COMPACT DESIGN
SIMPLIFIED MAINTENANCE

Available immediately as part of Sterwin Flour Service

FOR FAST, EFFICIENT and UNIFORM BLEACHING

For complete information write to:

Sterwin Chemicals Inc.

Subsidiary of Sterling Drug Inc.
1450 Broadway, New York 18, N. Y.
Flour Service Div., 8 West 9th St., Kansas City, Mo.

BRANCH OFFICES:
Atlanta, Buffalo, Evanston (Ill.), Dallas, Kansas City (Mo.), Los Angeles, Minneapolis, Portland (Ore.), St. Louis

SPECIALISTS IN FLOUR MATURING, BLEACHING AND ENRICHMENT

THE SARGENT

ELECTRIC DRYING OVEN . . . . . .

The Sargent electric drying oven was designed to fill the need of chemists for a dependable, low-cost, automatically controlled oven with a long service life. That it has achieved this objective is evidenced by the fact that thousands of these ovens are now in use in laboratories all over the world.

THE MULTIPLE CHROMEL WIRE HEATING ELEMENTS are arranged to give even heat distribution throughout the entire oven.

THE VENTILATING SYSTEM provides rapid transfer of air through the oven, which results in an exceptionally fast drying rate.

THE METAL WALLS are lined with ¼" Transite, to prevent excessive heat loss and the bimetallic thermostat maintains the heat in the oven to within ±1° C. of the desired temperature.

THE OPERATING RANGE is from slightly above room temperature to 200° C. (392° F.).

ALL CONTROLS—the three heat switch—the thermostat control and the pilot light—are located on the front panel which is actually the front of a drawer on which the heating elements are mounted. By removing two screws at each end of the panel the entire heating and control systems can be removed from the oven as a single unit.

MAXIMUM POWER CONSUMPTION 850 watts. Dimensions: Outside, 16" x 11¾" x 11⅞". Inside 9" x 11¼" x 11¼".

S-63995 OVEN—Sargent, Gravity Convection, Electric, Thermostatic, 200° C. With two metal shelves, thermometer (—10° C to 200° C in 1° subdivision), cord and plug for standard 115 volt, A.C. or D.C. circuits..... **$50.00**

S-64005 Ditto. But for operation from 230 volt, A.C. or D.C. circuits **$50.00**

SARGENT *IMMEDIATE SHIPMENT FROM STOCK*

NEW SARGENT CATALOG NOW AVAILABLE

SCIENTIFIC LABORATORY INSTRUMENTS · APPARATUS · SUPPLIES · CHEMICALS
E. H. SARGENT & COMPANY, 4647 W. FOSTER AVE., CHICAGO 30, ILLINOIS
MICHIGAN DIVISION, 1959 EAST JEFFERSON STREET, DETROIT 7, MICHIGAN
SOUTHWESTERN DIVISION, 5915 PEELER STREET, DALLAS 9, TEXAS

ON PY-RAN

Q. What is PY-RAN?

A. PY-RAN is the new, coated, anhydrous monocalcium phosphate leavening agent just put on the market by Monsanto.

Q. Just what is meant by "coated"?

A. Each granule of PY-RAN has a moisture-absorbent covering which protects it against premature reaction during storage or shelf life.

Q. What can PY-RAN do for me?

A. Give you leavening *stability* never before possible.

Q. How will this stability affect the shelf life of my products?

A. Aging tests conclusively prove PY-RAN's superior stability increases the life of prepared mixes and self-rising flour. The special coating retards premature reaction. Deteriorated, returned goods are reduced to a minimum.

Q. How will this greater stability affect the release of CO_2?

A. Greater stability of PY-RAN assures a controlled release of gas, even after long storage. Tests clearly prove PY-RAN releases CO_2 slowly during first two minutes of the mixing of the dough or batter. This two-minute reading is especially important as any CO_2 evolved during this time will be unavailable for leavening action in the oven.

Q. What other advantages has PY-RAN?

A. Slow, controlled release of gas as produced by PY-RAN gives excellent texture, volume, and crust and crumb color. Tunneling is virtually eliminated.

Q. Can PY-RAN be used in conjunction with other phosphate leavening agents?

A. Yes. PY-RAN will blend perfectly with other leavening phosphates such as Monsanto SAPP and HT phosphates.

Q. How can I find out more about PY-RAN?

A. We will be glad to furnish you samples so you can see for yourself what it will do. In addition, our completely equipped food laboratory is ready to work with you to show you the best way to use PY-RAN and other of the Monsanto leavening phosphates for maximum results. For more information or a copy of the new booklet, "MONSANTO PHOSPHATE LEAVENING AGENTS," write to MONSANTO CHEMICAL COMPANY, Inorganic Chemicals Division, 1700 South Second St., St. Louis 4, Mo.

Other Members of Monsanto's Leavening Family

Monsanto SAPP-40 . . . ideal for machine doughnut mixes

Monsanto SAPP-28 . . . a slow-action baking acid

Monsanto HT Phosphate . . . MCP monohydrate: use it alone or with other mixes for good results.

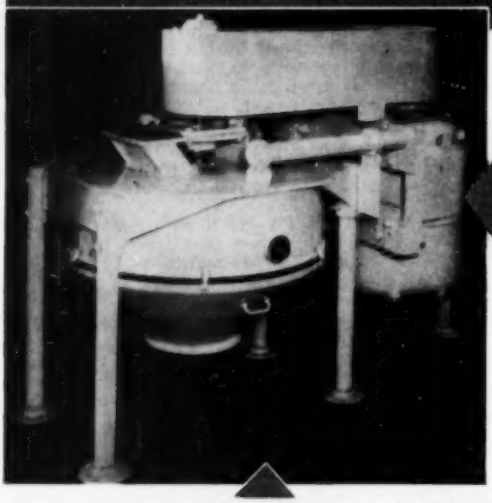
PY-RAN: Reg. U. S. Pat. Off.

MONSANTO

SERVING INDUSTRY...
WHICH SERVES MANKIND

500 CWT PER HOUR!!!

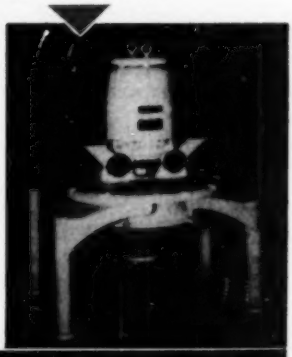
it's a cinch with the NEW "BIG INCH" . . . .



for Positive Infestation Control . . .

the NEW "ENTOLETER" 'BIG INCH' for 150 to 500 cwt per hour

the STANDARD "ENTOLETER" unit for 25 to 150 CWT per hour

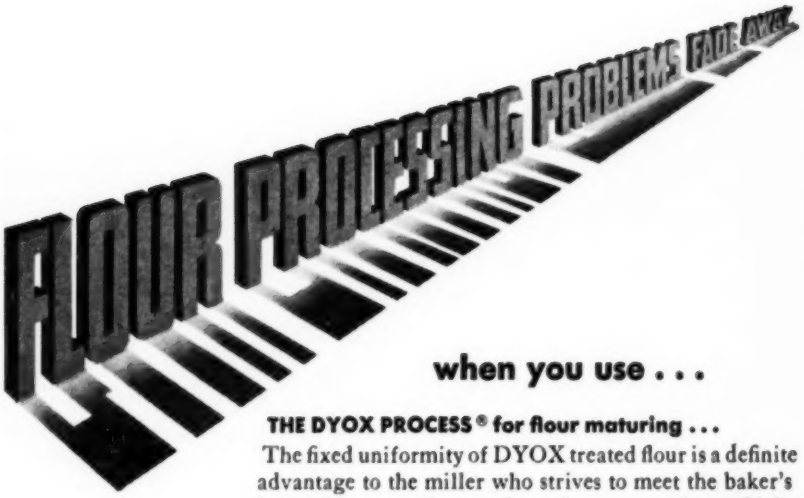


You guarantee your flour specifications... let "ENTOLETER" Infestation Control assure the complete cleanliness of all ingredients and finished products . . .

. . . the trademark "ENTOLETER" on milling equipment is your guarantee of complete satisfaction. Write to us for information concerning our products. ENTOLETER DIVISION, The Safety Car Heating and Lighting Company, Inc., 1153 Dixwell Ave., New Haven 4, Connecticut.

The trademark "ENTOLETER" is your guarantee of complete satisfaction

ENTOLETER
CENTRIFUGAL MACHINES



when you use . . .

THE DYOX PROCESS® for flour maturing . . .

The fixed uniformity of DYOX treated flour is a definite advantage to the miller who strives to meet the baker's demand for a starting product of constant value—which works in the bake shops with the least trouble and manipulation by the operator. DYOX treated flour assures overall dependability, uniformity of product, and ease of operation.

"NOVADELOX"® for whiter, brighter flour . . .

"Novadelox" is designed to meet the desire of the consuming public for a bread that is truly white—and to enable millers to produce a uniform product of standard color.

"N-RICHMENT-A"® for uniform enriching . . .

"N-Richment-A" provides a readily available, simple and troublefree product for enriching flour—with the assurance that the standard procedure of adding "N-RICHMENT-A" will produce a dependable standardized flour.

THE N-A FLOUR SERVICE DIVISION with more than a quarter-century of experience . . .

The N-A Flour Service Division with its skilled laboratories and staff is always available to work with you or your consultants on all phases of maturing, bleaching and enriching. Why not phone your nearest N-A Representative today!

DYOX
for flour maturing

NOVADELOX
for a whiter, brighter flour

N-Richment-A
for uniform enrichment

NA-62

NOVADEL FLOUR SERVICE DIVISION

WALLACE & TIERNAN INCORPORATED

BELLEVILLE 9, NEW JERSEY

REPRESENTATIVES IN PRINCIPAL CITIES